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IN VITRO PHYTOCHEMICAL, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY STUDIES ON *ALOCASIA SANDERIANA W.BULL*

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Abstract:

Objective: *This research is to investigate the phytochemical screening, antimicrobial and antioxidant activity of ethanolic leaf, stem and root tubers extracts of Alocasia sanderiana W.Bull.*

Methods: *Antimicrobial activity of ethanolic extracts of leaf, stem and root tubers of Alocasia sanderiana W.Bull was evaluated using agar well diffusion method against bacterial and fungal strains. Gentamicin 10µg/mL and Ketoconazole 10µg/mL were used as standards for antibacterial and antifungal assay respectively. Phytochemical screenings were done using standard methods. Antioxidant activity of ethanolic extracts of leaf, stem and root tubers of Alocasia sanderiana was evaluated by free radical scavenging activity on DPPH●, ABTS●+. The reducing power assay was evaluated against standard ascorbic acid.*

Results: *In the phytochemical study leaf, stem and roots tubers of plant parts were compared. The values show that leaf is more potential other then the stem and root. Leaf, stem and root extract showed significant in vitro antibacterial and antifungal activities. Antioxidant activity on comparing the phytochemical properties shows more potential other then the stem and root. Ethanolic extracts of leaf, stem and root tubers of Alocasia sanderiana showed significant in vitro antioxidant activity.*

Conclusion: *In phytochemical study, leaf shows more potential values compared to stem and root tubers. In antibacterial study, stem shows more inhibition than leaf and root. In antifungal study, stem and root show more inhibition than leaf. Antioxidant activity studies of leaf shows more potential compared to stem and root tubers of A.Sanderiana due to presence of various phytoconstituents and it could be a source of new compounds.*

Keywords: *Phytochemical study, antimicrobial activity, Araceae, Antioxidant activity.*

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INTRODUCTION:

Alocasia sanderiana W.Bull is a plant in the Araceae family. Alocasia Sanderiana W.Bull is also known as the kris plant because of the resemblance of its leaf edges to the wavy blade of the kalis dagger (also known as kris plant). It is a tropical perennial with upright shiny, V-shaped and deeply lobed leaves. The plant can be up to 6 ft (2m) tall and large in its native habitat. However, cultivated specimens are smaller. It possesses leaves that are evergreen, pelted, V-shaped, deeply lobed, and a glossy deep-green with large silvery white veins. They are about 12-16 in (30-40cm) long and 6-8 in (15-20cm) wide, with red-green undersides. The leaf stem is about 2 ft (60cm) long. The rhizome of *A.Sanderiana* is vertically placed and is known as root stock. Female flowers are grouped at the lower part of the inflorescence, whereas the male flowers are at the top. According to literature report, alocasia is a kris plant native to tropical and subtropical Asia to Eastern Australia. Alocasia genus consists of about 79 species of which 28 are cultivated variety. *Alocasia sanderiana W.Bull* plant extract used in nanosilver particles to fight and prevent bacteria in vitro [1] and *Alocasia sanderiana W.Bull* endemic plant available in Tamilnadu[2,3,4], India [5]. The various species of alocasia plats are used in the treatment of dysentery, leucorrhoea and they have anti-inflammatory, wound healing [6], cytotoxic [7,8,9], antimicrobial [10,11,12,13,14], Antioxidant [15,16,17,18,19], anti-diabetic [20,21], anticancer [22] and antitumor properties [23,24]. Selection of this plant is study of phytochemical, antioxidant, antimicrobial, anti-inflammatory and antidiabetic activity of different parts like leaf, stem, and root tubers of *alocasia sanderiana W.Bull* plant. The aim of the present study is to evaluate the phytochemical analysis, antimicrobial and antioxidant activity of different parts like leaf, stem, and root tubers of *alocasia sanderiana* plant.

MATERIALS AND METHODS:

Materials

All chemicals and solvents are of analytical reagent grade and procured from HI MEDIA and SD FINE chemicals. The healthy and disease free plant parts leaf, stem and root tubers of *alocasia sanderiana* were collected from southern region of Coimbatore Tamilnadu, India, in the month of January 2012. The botanical identification was authenticated by a botanist. The fresh plant parts of each leaf stem and root tubers were washed with tap water and then rinsed with distilled water. Washed plant material was air dried in the laboratory at room temperature for 5-8 days or until they were easily broken by hand. Once completely dried, plant parts were grounded to a fine powder using an electronic blender. Plants were stored in a closed container at room temperature until required.

Preparation of Ethanolic Crude Extract

The powdered plant parts leaf, stem and root of each material were mixed with sufficient quantity of ethanol solvent. It was kept in rotary shaker at 100rpm for 48 hrs. At the end of 48 hrs, each extract was filtered through Whatman No.1 filter paper and the filtrates were concentrated at room temperature in order to reduce the volume. The paste like extracts were stored in pre-weighed screw capped bottles and the yield of extracts were weighed. These screw scrapped bottles were kept in refrigerator at 4 °C for future usage. Each extract was individually reconstituted using minimal amounts of the extracting solvent prior to use.

Qualitative Phytochemical Screening

The phytochemical screening of the sample was carried out as described by Nweze et al., (2004)[25] and Senthilkumar and Reetha (2009)[26]. The samples were screened for carbohydrates, alkaloids, flavonoids, steroids, phenols, tannins, saponin, glycosides, proteins, terpenes and amino acids. Phytochemical study results are tabulated in table 1.

Antimicrobial Activity

Preparation of Inocula for Antibacterial Activity

The test organisms *Staphylococcus aureus* (MTCC3381), *Bacillus cereus* (MTCC430), *Pseudomonas aeruginosa* (MTCC424), *Klebsiella pneumonia* (MTCC432) and *Escherichia coli* (MTCC739) were subcultured by streaking them on nutrient agar [28], followed by incubation for 24 h at 37 °C. Several colonies of each bacterial species were transferred to sterile nutrient broth. The suspensions were mixed for 15 sec and incubated for 24 hrs at 37 °C on an orbital incubator shaker. Working concentration of the microbial suspension was prepared in 3 mL of sterile saline to turbidity equivalent to 0.5 McFarland scale (i.e., adjusting the optical density to 0.1 at 600 nm), yielding a cell density of 1-2 × 10^5 CFU/mL.

Antibacterial Activity

Nutrient Agar (NA) plates were seeded with broth culture of different bacteria. In each of these plates, wells were cut out using sterile cork borer. Using sterilized dropping pipettes, different concentrations (1000, 2000 and 3000 µg/well) of leaf, root plant extract and (250, 500 and 750 µg/well) of stem extract was carefully added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were then incubated at 37 °C for 18–24 hrs. Gentamicin (10 µg) was used as positive control and DMSO as negative control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. Results are tabulated in table 2.

Preparation of Inocula for Antifungal Activity

The fungal pathogens *Candida albicans* (MTCC227), *Fusarium solani* (MTCC2935), *Aspergillus fumigates* (MTCC343), *Rhizopus*

oryzae (MTCC262) and *Aspergillus terreus* (MTCC1281) were cultured in Sabouraud dextrose agar for 72 hrs at 27 °C and the spores/cells were harvested in sterile saline using a sterile squirrel brush [27]. Working concentration of spore suspension was prepared with sterile saline to turbidity equivalent to 0.5 McFarland scale (i.e., adjusting the optical density to 0.1 at 530 nm), yielding a cell density of 1-5 × 10^6 CFU/mL.

Antifungal Activity

Sabouraud dextrose agar plates were seeded with one hundred microliter of spore/cell suspension of different test organisms. In each of these plates, wells were cut out using sterile cork borer. Using sterilized dropping pipettes, different concentrations (1000, 2000 and 3000 µg/well) of plant extract was carefully added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were then incubated at 27 °C for 48 hrs. Ketoconazole (10 µg) was used as positive control and DMSO as negative control. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. Results are tabulated in table 3.

Antioxidant Activity

Free Radical Scavenging Activity on DPPH●

Principle:

The scavenging reaction between (DPPH.) and an antioxidant (H-A) can be written as:

(DPPH) + (H-A) ⟶ DPPH-H + (A)
(Purple) (Yellow)

Antioxidants react with (1,1-diphenyl-2-picrylhydrazyl), which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance of DPPH radical decreased. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Chemicals Required:

1.0.1 Mm solution of DPPH in methanol was prepared and used in the study.

2. Ascorbic acid (1%)

Procedure

The antioxidant activity [28] of the sample leaf, stem and root tubers was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois (1958). The sample extracts at various concentrations (100 - 500µg/mL) were taken and the volume was adjusted to 100 µl with methanol. Methanolic solution (5 ml of 0.1 mM) of DPPH• was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Ascorbic acid at various concentrations (10 to 50µg/mL) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Percentage radical scavenging activity of the sample was calculated as follows:

% DPPH radical scavenging activity = (Control OD-sample OD / Control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC50) under the assay condition was calculated from the graph of inhibition percentage against sample concentration. Results are presented in Table 4.

Free Radical Scavenging Activity on ABTS●+

The antioxidant activity [29] of the samples leaf, stem and root tubers was measured by ABTS radical cation decolorization assay according to the method of Re *et al*. (1999). ABTS●+ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30 ºC to give an absorbance at 734 nm of 0.700 ± 0.02. The stock solution of the sample extracts were diluted such that after introduction of 10 µl aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 ml of diluted ABTS solution to 10 µl of sample (100-500 µg/mL), absorbance was measured at 734 nm at exactly 30 min after the initial mixing. Samples were analyzed in triplicate. Percentage radical scavenging activity of the sample was calculated as follows:

% ABTS Radical Scavenging Activity = (Control OD-sample OD / Control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration. Results are presented in Table 5.

Reducing Power Assay

Principle:

The reducing power of ethanolic extract of alocasia was determined by the slight modification of the method of Oyaizu, [30]. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride ⟶ Potassium ferricyanide + ferrous chloride

Chemicals Required

Potassium ferricyanide (1% w/v), phosphate buffer (0.2 M, pH 6.6), trichloro acetic acid (10%), ferric chloride (0.1%) and ascorbic acid (1%).

Phosphate Buffer Preparation

Dibasic sodium phosphate (37.50 ml of 0.2M) is mixed with 62.5 ml monobasic sodium phosphate and diluted to 100 ml with water.

Protocol for Reducing Power

Various concentrations of the plant leaf, stem and root tubers extracts (100,200,300,400, 500µg/mL) in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations (100,200,300,400, 500µg/mL) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. Reducing power was measured by varying the concentration of the extract and the contact time. Results are presented in Table 6.

RESULTS AND DISCUSSION:

In consideration of the importance of phytochemical and antimicrobial investigation on endemic plants, *A. Sanderiana* was chosen for the present work. The result of the study and the discussion pertaining to it is presented below.

Phytochemical Screening

The phytoconstituents of leaf stem and root tubers of A.Sanderiana were investigated using ethanolic extracts and the results are summarized in Table 1.

Phytochemical Screening of Leaf Extract

The preliminary phytochemical screening of the ethanolic leaf extracts of *A. Sanderiana* revealed strong presence of various chemical substances such as tannins, flavonoids, phenols and steroids, moderate presence of glycosides. Trace amount of saponins, terpenes, carbohydrates and proteins are present. Alkaloids and amino acids are not present.

Phytochemical Screening of Stem Extract

The preliminary phytochemical screening of the ethanolic stem extracts of *A. Sanderiana* revealed strong presence of secondary metabolites such as tannins, moderate presence of flavonoids and phenols. Trace amounts of saponins, terpenes, carbohydrates, proteins, glycosides and steroids are present. Alkaloids and amino acids are not present.

Phytochemical screening of root extract

The preliminary phytochemical screening of the ethanolic root tubers extracts of *A. Sanderiana* revealed moderate presence of tannins, trace amounts of glycosides, steroids, saponins, terpenes, alkaloids, flavonoids, carbohydrates, proteins and phenols. Amino acids are not present.

Table 1: Phytochemical Screening of Leaf, Stem and Root Ethanolic Extracts

S.No	Secondary Metabolites	Leaf	Stem	Root
1	Tannins	+++	+++	++
2	Glycosides	++	+	+
3	Saponins	+	+	+
4	Terpenes	+	+	+
5	Flavonoids	+++	++	+
6	Alkaloids	-	-	+
7	Carbohydrates	+	+	+
8	Steroids	+++	+	+
9	Proteins	+	+	+
10	Phenols	+++	++	+
11	Amino acids	-	-	-

+++ - Strongly positive ++ - Moderately positive

\+ - Trace – - Not detected

‘

Antimicrobial Activity [Antibacterial Activity]

Antimicrobial activity of ethanolic extracts of leaf, stem and roots tubers of A. Sanderiana were analyzed against ten clinically significant organisms using disc diffusion method (Table 2, 3). All the extracts tested showed a measurable zone of inhibition.

Antibacterial Activity of Leaf Extracts

Antibacterial activities of ethanolic extracts of leaf were analyzed against five clinically significant organisms using disc diffusion method (Table 2). The leaf extracts of different concentrations (1000, 2000 and 3000 µg/well) test showed a measurable zone of inhibition. The standard positive control showed inhibition diameter ranging from 22-23 mm (Gentamicin 10 µg) against the tested organisms. *Staphylococcus aureus*, *escherichia coli*, *klebsiella pneumonia*, *pseudomonas aeruginosa*, and *bacillus cereus* were tested with plant leaf extracts. *Pseudomonas aeruginosa* shows higher zone of inhibition compared with other test organisms (concentration 1000, 2000 and 3000 µg/well, zone of inhibition 31.00±0.00, 33.33±1.55, 34.67±0.58%) other four test organisms showed in the rage of 10-13 mm zone of inhibition with different concentrations. Increase in the plant extract concentration (1000, 2000 and 3000 µg/well) increases zone of inhibition. This indicates that plant leaf contains active bio marker compounds.

Antibacterial Activity of Stem Extracts

Antibacterial activities of ethanolic extracts of stem were analyzed against five clinically significant organisms using disc diffusion method (Table 2). The stem extracts of different concentrations (250, 500 and 750 µg/well) showed a measurable zone of inhibition. The standard positive control showed inhibition diameter ranging from 22-23 mm (Gentamicin 10 µg) against the tested organisms. *Staphylococcus aureus*, *escherichia coli*, *klebsiella pneumonia*, *pseudomonas aeruginosa*, and *bacillus cereus* were tested with plant stem extracts. *Staphylococcus aureus* shows higher zone of inhibition compared with other test organisms (concentration 250, 500 and 750 µg/well, zone of inhibition 32.33±1.15, 35.67±0.58, 38.33±0.58%) . Other test organisms showed in the rage of 10-16 mm zone of inhibition with different concentrations. 250 µg/well plant extracts did not show any zone of inhibition against escherichia coli, klebsiella pneumonia, and bacillus cereus. When plant extract concentration (250, 500 and 750µg/well) is increased, zone of inhibition also increases. This indicates that the plant stem contains active bio marker compounds.

Antibacterial Activity of Root Extracts

Antibacterial activities of ethanolic extracts of the root were analyzed against clinically significant organisms viz. *staphylococcus aureus*, *escherichia coli*, *klebsiella pneumonia*, *pseudomonas aeruginosa*, and *bacillus cereus* using disc diffusion method (Table 2). The standard positive control showed inhibition diameter ranging from 26-28mm (Gentamicin 10µg) against the tested organisms. *Staphylococcus aureus*, *escherichia coli*, *klebsiella pneumonia*, *pseudomonas aeruginosa*, and *bacillus cereus* tested with plant root extracts of different concentrations (1000, 2000 and 3000µg/well) showed only the inhibition of *pseudomonas aeruginosa* (3000 µg/well, 10.00±0.00), other four test organs did not showi zone of inhibition against plant root extract. It indicates that plant root does not contain antibacterial active bio marker compounds.

Table 2: Antibacterial Activity of *Alocasia Sanderiana* Ethanolic Extracts

Plant parts	Concentration (µg)	Zone of inhibition (mm)*				
		Staphylococcus aureus	Escherichia coli	Klebsiella pneumonia	Pseudomonas aeruginosa	Bacillus cereus
Leaf extract	1000	10.67±0.58	10.00±0.00	11.33±0.58	31.00±0.00	10.00±0.00
	2000	12.33±0.58	11.33±0.58	12.67±0.58	33.33±1.55	11.33±0.58
	3000	13.33±0.58	12.33±0.58	13.67±0.58	34.67±0.58	12.67±0.58
	Gentamici 10 µg	23.00±0.00	22.00±0.00	22.67±0.58	22.00±0.00	22.33±0.58
Stem extract	250	32.33±1.15	-	-	10.00±0.00	-
	500	35.67±0.58	10.00±0.00	10.00±0.00	13.00±0.00	12.00±0.00
	750	38.33±0.58	14.00±0.00	13.00±0.00	16.00±0.00	14.00±0.00
	Gentamicin10 µg	22.67±0.58	22.33±0.58	23.33±0.58	24.00±0.00	23.33±0.58
Root extract	1000	-	-	-	-	-
	2000	-	-	-	-	-
	3000	-	-	-	10.00±0.00	-
	Gentamicin 10 µg	28.00±0.00	26.00±0.00	28.00±0.00	28.00±0.00	26.00±0.00

**Values are means of three independent analysis ± Standard Deviation (n=3)*

Antifungal Activity

Antifungal activity of ethanolic extracts of leaf, stem and roots of *A. Sanderiana* were analyzed against five clinically significant organisms using disc diffusion method (Table 3). All the extracts tested showed a measurable zone of inhibition. The standard positive control showed inhibition diameter ranging from 12-22mm (Ketoconazole 10µg) against the tested organisms.

Antifungal Activity of Leaf Extracts

Antifungal activities of ethanolic leaf extracts of *A. Sanderiana* were analyzed against a few clinically significant organisms viz. *fusarium solani*, *aspergillus fumigates*, *aspergillus terreus*, *rhizopus oryzae*, and *candida albicans* using disc diffusion method. The standard positive control showed inhibition diameter ranging from 12-22mm (Ketoconazole 10µg) against the tested organisms. *fusarium solani*, *aspergillus fumigates*, *aspergillus terreus*, *rhizopus oryzae*, and *candida albicans* were tested with plant leaf extracts of different concentrations (1000, 2000 and 3000 µg/well). The test showed only the inhibition of *candida albicans* (21.67±0.58, 26.67±0.58, 30.00±0.58). Other four test organisms did not show zone of inhibition against the plant leaf extract. It indicates that the plant root does not contain antifungal active bio marker compounds.

Antifungal Activity of Stem Extracts

Antifungal activities of ethanolic stem extracts of *A. Sanderiana* were analyzed against clinically significant organisms like *fusarium solani, aspergillus fumigates, aspergillus terreus, rhizopus oryzae, and candida albicans* using disc diffusion method. The standard positive control showed inhibition diameter ranging from 12-15 mm (Ketoconazole 10µg) against the tested organisms. The organisms were tested with plant stem extracts of different concentrations (1000, 2000 and 3000 µg/well). The test showed inhibition of *Fusarium solani* (11.33±0.58, 14.67±0.58, 18.67±0.58) and *Candida albicans* (21.67±0.58, 26.67±0.58, 30.00±0.58). The other three test organisms did not show any zone of inhibition against plant stem extracts.

Antifungal Activity of Root Extracts

Antifungal activities of ethanolic root extracts of *A. Sanderiana* were analyzed against clinically significant organisms such as *fusarium solani, aspergillus fumigates, aspergillus terreus, rhizopus oryzae, and candida albicans* using disc diffusion method. The standard positive control showed inhibition diameter ranging from 12-14 mm (Ketoconazole 10µg) against the tested organisms. The organisms were tested with plant root extracts of different concentrations 1000, 2000 and 3000µg/well. The test shows inhibition of candida albicans, aspergillus terreus and rhizopus oryzae. The other two test organisms did not show zone of inhibition against plant root extracts.

Table 3: Antifungal Activity of Alocasia Sanderiana Ethanolic Extracts

Plant parts	Concentration (µg/well)	Zone of inhibition (mm)*				
		Fusarium solani	Aspergillus fumigates	Aspergillus terreus	Rhizopus oryzae	Candida albicans
Leaf extract	1000	-	-	-	-	21.67±0.58
	2000	-	-	-	-	26.67±0.58
	3000	-	-	-	-	30.00±0.58
	Ketoconazole 10 µg	15.00±0.00	14.33±0.00	12.00±0.00	15.00±0.00	22.00±0.00
Stem extract	1000	11.33±0.58	-	-	-	17.67±0.58
	2000	14.67±0.58	-	-	-	25.00±0.00
	3000	18.67±0.58	-	-	-	29.67±0.58
	Ketoconazole 10 µg	12.00±0.00	12.00±0.00	13.00±0.00	12.33±0.58	14.67±0.58
Root extract	1000	-	-	-	-	19.00±0.00
	2000	-	-	12.30±0.60	15.70±0.60	21.30±0.60
	3000	-	-	16.00±0.00	19.00±0.00	24.70±1.20
	Ketoconazole 10 µg	12.00±0.00	12.00±0.00	12.00±0.00	14.00±0.00	12.00±0.00

**Values are means of three independent analysis ± Standard Deviation (n=3)*

In consideration of the importance of antioxidant activity, investigation on *A. Sanderiana* was carried out in the present work. The result of the study and the discussion pertaining to it is presented below.

Antioxidant Activity

The DPPH radical scavenging potentials of leaf, stem and root tubers extracts of *A.Sanderiana* are presented in Table 4. The ethanolic leaf, stem and root extract DPPH radical scavenging capacity was found to be comparable to ascorbic acid.

DPPH Radical Scavenging Activity of Leaf Extracts

The DPPH radical scavenging capacity of leaf extracts of *A.Sanderiana* leaves are presented in Table 4 and Figure 1. The ethanolic leaf extract at 100, 200, 300, 400, 500 µg/ml exhibited DPPH radical scavenging activity comparable to ascorbic acid (Figure 2). The DPPH scavenging activities of the extracts are expressed as IC_{50} value. The ethanolic leaf extracts (IC_{50}= 84.75±0.47 µg/mL) showed the strongest radical scavenging activity (Figure 3).

DPPH scavenging is widely used to test the free radical scavenging activity of several natural products [31]. This radical scavenging activity of extracts could be related to the nature of phenolic compounds, thus contributing to their electron transfer/hydrogen-donating ability. The result indicates that medicinal plants have significant effects on scavenging free radicals. The better DPPH scavenging activity may be related to the higher phenolic contents [32]. The reduction mechanism of the DPPH radical correlates with the hydroxyl groups on the antioxidant molecule[33], so the mechanism might involve the delocalization of an electron onto the p-substituted OH-group on the molecule prior to the donation of a second hydrogen to reduce DPPH, which also depends on the stability and reaction potential of the molecular structure [34].

DPPH Radical Scavenging Activity Of Stem Extracts

The DPPH radical scavenging capacity of stem extracts of *A.Sanderiana* is presented in Table 4 and Figure 1. The ethanolic stem extract exhibited DPPH radical scavenging activity was found to be comparable to ascorbic acid (Figure 2). The DPPH scavenging activities of the extracts are expressed as IC_{50} values. The ethanolic stem extracts (IC_{50}= 129.31±0.15 µg/mL) showed the strongest radical scavenging activity (Figure 3).

Table 4: DPPH Radical Scavenging Assay of A. Sanderiana Ethanolic Extracts

S.No	Sample Extract concentration (µg)	Percentage of inhibition activity (%)			Ascorbic acid standard concentration (µg)	Standard ascorbic acid % inhibition
		Leaf extract	Stem extract	Root extract		
1	**100**	42.10±0.45	30.00±0.22	20.88±0.23	**10**	47.56±0.81
2	**200**	53.84±0.15	42.22±0.33	31.22±0.29	**20**	59.98±0.71
3	**300**	56.56±0.25	47.67±0.15	36.36±0.27	**30**	68.83±0.54
4	**400**	67.29±0.57	58.01±0.55	39.41±0.34	**40**	79.09±0.53
5	**500**	70.00±0.15	62.55±0.25	48.44±0.38	**50**	88.58±0.67
6	**IC_{50} (µg/mL)**	84.75±0.47	129.31±0.15	193.11±11.75	**IC_{50} (µg/mL)**	9.70±0.03

**Values are means of three independent analysis ± Standard Deviation (n=3)*

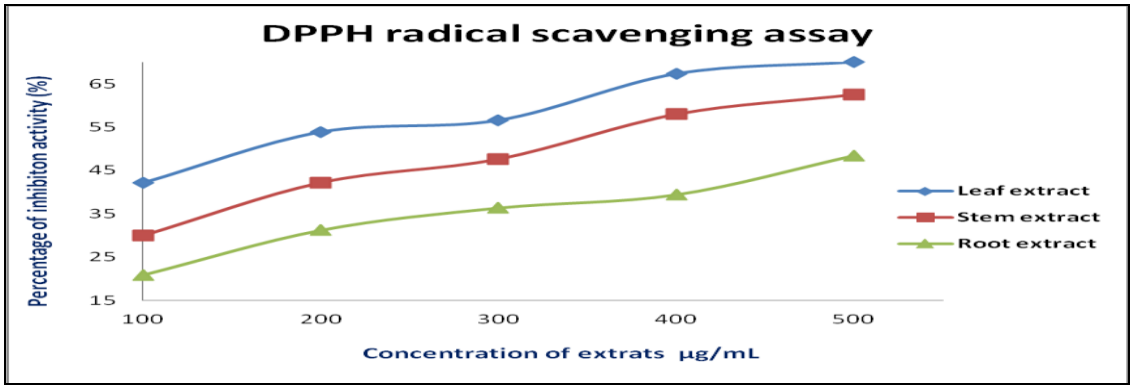

Fig1: Comparison of DPPH Radical Scavenging Assay

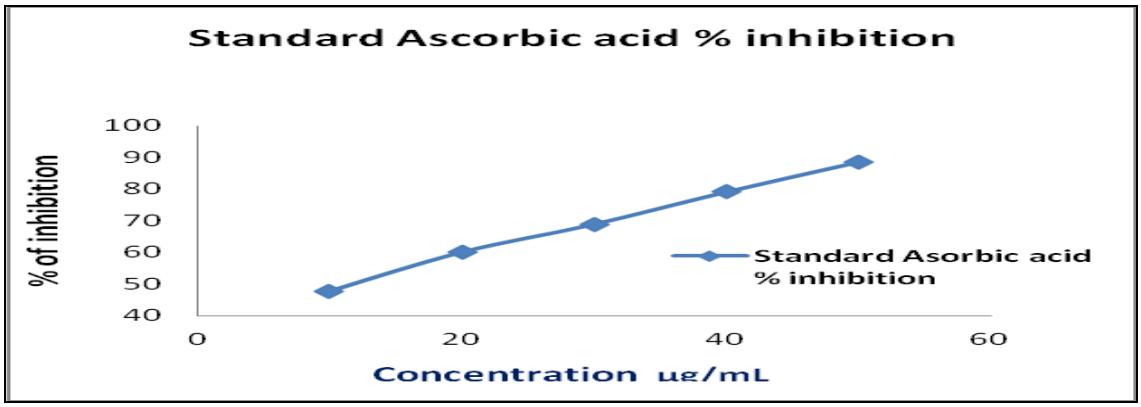

Fig 2: Standard Ascorbic acid DPPH Radical Scavenging Activity

DPPH Radical Scavenging Activity of Root Extracts

The DPPH radical scavenging capacity of root extracts of *A.Sanderiana* are presented in Table 4 and Figure 1. The ethanolic root extract exhibited DPPH radical scavenging activity comparable to ascorbic acid (Figure 2). The DPPH scavenging activities of the extracts are expressed as IC_{50} values. The ethanolic root extracts (IC_{50}= 193.11±11.75 µg/mL) strongest radical scavenging activity (Figure 3).

Plant extracts having less IC_{50} value indicate that the extracts have more antioxidant activity. Among the three samples, leaf extract showed more antioxidant activity than stem and root extracts.

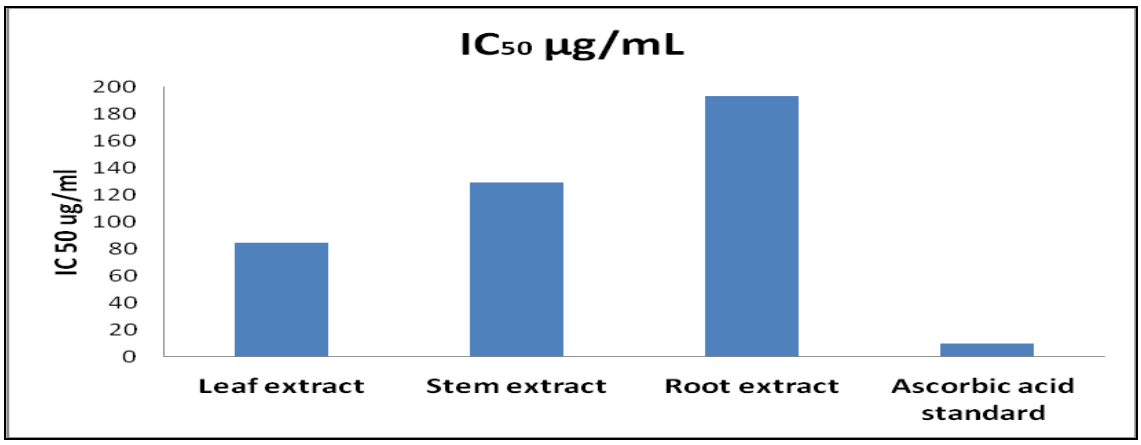

Fig 3: Comparison of DPPH Radical Scavenging Activity IC_{50} Values with Plant Extracts

ABTS Free Radical Scavenging Activity

The ABTS Free radical scavenging activity of leaf, stem and root extracts of A.Sanderiana are presented in Table 5. The ethanolic leaf, stem and root extract ABTS Free radical scavenging potential was found to be comparable to ascorbic acid.

ABTS Free Radical Scavenging Activity of Leaf Extracts

The ABTS free radical scavenging activity of leaf extracts of *A.Sanderiana* leaves are presented in Table 5 and Figure 4. The ethanolic leaf extract exhibited ABTS radical scavenging activity comparable to ascorbic acid (Figure 5). The ABTS scavenging activities of the extracts are expressed as IC_{50} values. The ethanolic leaf extracts (IC_{50}= 98.62±0.25 µg/mL) showed strongest radical scavenging activity (Figure 6).

ABTS Free Radical Scavenging Activity of Stem Extracts

The ABTS Free radical scavenging capacity of stem extracts of *A.Sanderiana* are presented in Table 5 and Figure 4. The ethanolic stem extract exhibited ABTS radical scavenging activity comparable to ascorbic acid comparable to ascorbic acid (Figure 5). The ABTS scavenging activities of the extracts are expressed as IC_{50} values. The ethanolic stem extracts (IC_{50}= 159.11±0.72 µg/mL) showed strongest radical scavenging activity (Figure 6).

Table 5: ABTS Free Radical Scavenging Activity of A.Sanderiana Ethanolic Extracts

S.No	Sample Extract concentration (µg/mL)	Percentage of inhibition activity (%)			Ascorbic acid standard concentration (µg/mL)	Standard Ascorbic acid % inhibition
		Leaf extract	Stem extract	Root extract		
1	**100**	35.57±0.95	22.70±0.11	10.06±0.67	**10**	32.76±3.76
2	**200**	65.86±0.86	39.21±0.17	18.39±0.10	**20**	51.21±3.85
3	**300**	86.21±1.07	51.22±0.55	31.00±2.60	**30**	67.66±4.07
4	**400**	89.97±0.08	64.17±1.02	39.22±1.68	**40**	84.26±0.84
5	**500**	90.97±0.22	73.62±0.81	50.89±1.64	**50**	99.33±0.08
6	**IC_{50} (µg/mL)**	98.62±0.25	159.11±0.72	223.00±3.43	**IC_{50} (µg/mL)**	9.39±0.15

**Values are means of three independent analysis ± Standard Deviation (n=3)*

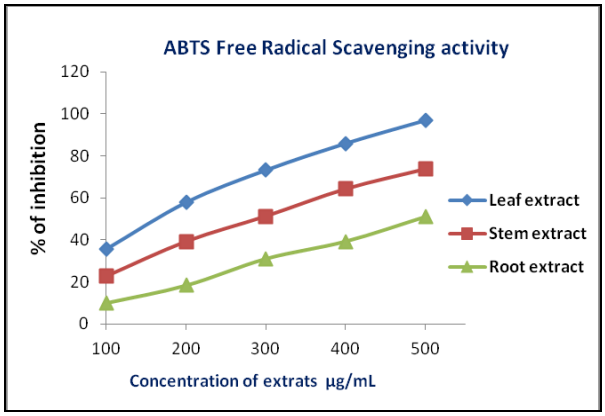

Fig4: Comparison of ABTS Free Radical Scavenging Activity

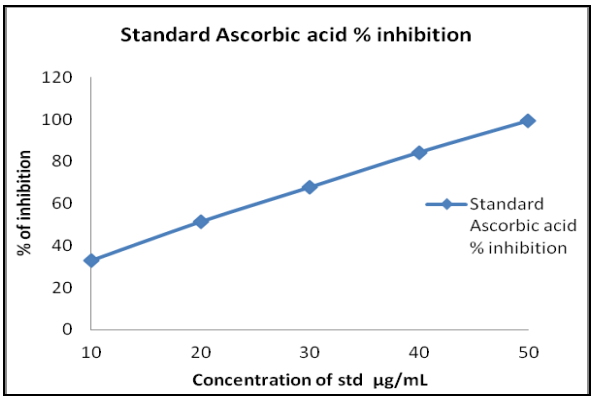

Fig 5: Standard Ascorbic acid ABTS Free Radical Scavenging Activity

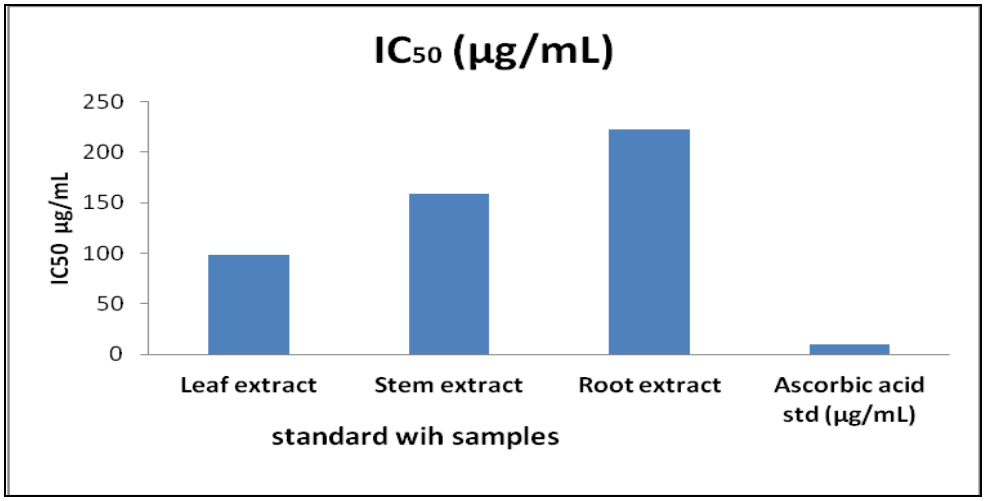

Fig 6: Comparison of ABTS Free radical scavenging activity IC_{50} values with plant extracts

ABTS Free Radical Scavenging Activity of Root Extracts

The ABTS Free radical scavenging activity of root extracts of *A.Sanderiana* are presented in Table 5 and Figure 4. The ethanolic root extract exhibited ABTS radical scavenging activity comparable to ascorbic acid (Figure 5). The ABTS scavenging activities of the extracts are expressed as IC_{50} values. The ethanolic root extracts (IC_{50}= 223.00±3.43 µg/mL) showed strongest radical scavenging activity (Figure 6).

Plant extracts having less IC_{50} value it's indicating that extracts having more antioxidant activity. Among the three sample leaf extract showing more antioxidant activity than stem and root extracts.

Reducing Power Activity

In this assay, the yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each compound. The presence of radicals (i.e antioxidant) causes the conversion of the Fe^{3+}/ ferricyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls of Prussian blue at 700 nm [5] we can monitor the Fe^{2+} concentration; a higher absorbance at 700 nm indicates a higher reducing power. The results of the reducing power activity of *A.Sanderiana* ethanolic extracts assay are given in Table 6.

Table 6: Reducing Power Activity of *A.Sanderiana* Ethanolic Extracts

S.No	Sample Extract concentration (µg/mL)	Absorbance at 700nm			
		Leaf extract	Stem extract	Root extract	Ascorbic acid standard
1	100	0.140±0.021	0.101±0.007	0.083±0.041	0.211±0.081
2	200	0.231±0.007	0.192±0.025	0.152±0.017	0.410±0.090
3	300	0.397±0.027	0.294±0.009	0.202±0.008	0.610±0.011
4	400	0.540±0.013	0.405±0.016	0.310±0.044	0.797±0.081
5	500	0.664±0.023	0.487±0.022	0.394±0.081	0.971±0.075

**Values are means of three independent analysis ± Standard Deviation (n=3)*

Reducing Power Activity of Leaf Extracts

The reducing power capacity of leaf extracts of *A.Sanderiana* are presented in Table 6 Figure 7. The ethanolic leaf extract showed better reducing power comparable to standard ascorbic acid (Figure 7).

Reducing Power Activity of Stem Extracts

The reducing power capacities of stem extracts of *A.Sanderiana* are presented in Table 6 Figure 7. The ethanolic leaf extract showed better reducing power comparable to standard ascorbic acid (Figure 7) comparable to ascorbic acid.

Reducing Power Activity of Root Extracts

The reducing power capacities of root extracts of *A.Sanderiana* are presented in Table 6 and Figure 7. The ethanolic root extract showed better reducing power comparable to standard ascorbic acid (Figure 7).

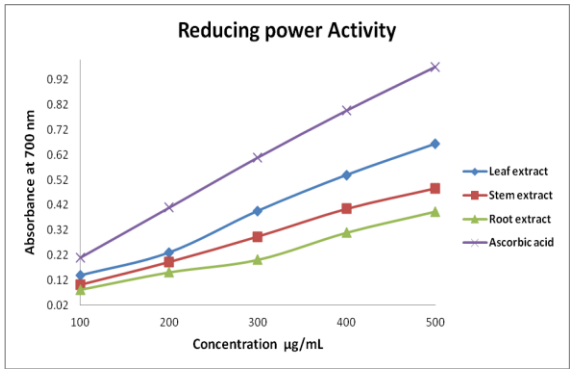

Fig 7: Comparison of Reducing Power Activity

The higher absorbance of leaf extracts may be due to its strong reducing power potential. Comparing leaf, stem and root tubers extracts, the leaf extracts showed better reducing power than stem and root extracts. The reducing power of the extracts may be due to the biologically active compounds in the extract which possess potent donating abilities. This assay further confirmed the antioxidant properties of the extracts.

CONCLUSION

In the present study, the preliminary phytochemical screening of the ethanolic leaf extracts of *A. Sanderiana* revealed strong presence of tannins, flavonoids, phenols and steroids, moderate presence of glycosides. Trace amount of saponins, terpenes, carbohydrates and proteins are also present. Stem extracts revealed strong presence of tannins, moderate presence of flavonoids and phenols. Trace amount of saponins, terpenes, carbohydrates, proteins, glycosides and steroids are also present. Alkaloids and amino acids are not present in leaf and stem. Root extracts revealed moderate presence of tannins, trace amounts of glycosides, steroids, saponins, terpenes, alkaloids, flavonoids, carbohydrates, proteins and phenols. Amino acids are not present. When all the three plant parts are compared, leaf shows more potential than the stem and root. These activities may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins, steroids, and phenols.

Antibacterial activities of ethanolic extracts of leaf, stem and root were analyzed against five clinically significant organisms viz. *staphylococcus aureus*, *escherichia coli*, *klebsiella pneumonia*, *pseudomonas aeruginosa* and *bacillus cereus* using disc diffusion method. The standard positive control showed inhibition diameter ranging from 26-28 mm (Gentamicin 10 µg) against the tested organisms. Leaf extracts (1000, 2000, 3000 µg/well concentration) were found to be effective in inhibiting the growth of *pseudomonas aeruginosa* compared with other bacteria. Stem extracts (250, 500, 750 µg/well concentration) were found to be effective in inhibiting the growth of staphylococcus aureus compared with other bacteria. Root extracts (1000, 2000, 3000 µg/well concentration) were not found to be effective in inhibiting the growth of any bacteria. Compared to leaf and root, stem shows more inhibition against bacterial growth.

Antifungal activities of ethanolic extracts of leaf, stem and root tubers were analyzed against five clinically significant organisms viz. *fusarium solani*, *aspergillus fumigates*, *aspergillus terreus*, *rhizopus oryzae*, *candida albicans* using disc diffusion method. The standard positive control showed inhibition diameter ranging from 12-22 mm (Ketoconazole 10 µg) against the tested organisms. The test with different concentrations (1000, 2000 and 3000µg/well) of leaf extracts showed only the inhibition of *candida albicans* (21.67±0.58, 26.67±0.58, 30.00±0.58%) The test with stem extracts of different concentrations (1000, 2000 and 3000 µg/well) showed inhibition of *fusarium solani* and *candida albicans*. The test with different concentrations (1000, 2000 and 3000µg/well) of root extracts test shows inhibition of *candida albicans*, *aspergillus terreus* and *rhizopus oryzae*. Compared to leaf, stem and root tubers show more inhibition of fungal growth.

Among the three samples, leaf extract shows more antioxidant activity (DPPH and ABTS) than stem and root extracts. Comparing leaf, stem and root tubers extracts, the leaf extracts showed better reducing power than stem and root extracts. The reducing power of the extracts may be due to the biologically active compounds in the extract which possess potent donating abilities. From the present investigations, we can conclude that *A.sanderiana* possesses significant antimicrobial activity due to the presence of various phytoconstituents and it could be a source of new compounds. Ethanolic extracts of leaf, stem and root tubers of *A.Sanderiana* showed *in vitro* anti-inflammatory

and antidiabetic activities. From the ethanolic extracts of *A.sanderiana*, two new compounds have been isolated. The characterization and structural conformation of the new compounds are in progress.

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