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An In Vitro Study: Antioxidant, Phytochemical Analysis and Antimicrobial Activities of *Achranthes aspera*

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Abstract: *Medicaments, plants and plant-based are the basis of many of the modern pharmaceuticals we use today for our various purposes. The aim of the present study was to evaluate the antioxidant, phytochemical and antibacterial and antifungal activities of the Achyranthes aspera plant extract in different organic solvents. The radical scavenging activity of the different extracts of root, stem, leaf, and seed were evaluated by DPPH assay and the antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa, and antifungal activity against Fusarium sp. and Aspergillus nigerwas studied by Agar well cut diffusion method. All of the extracts exhibited different antioxidant and antibacterial activities and the activities varied from solvent to solvent, and the activities are concentrated. The antioxidant and antimicrobial activities were compared with the positive control Ascorbic acid and Cefuroxime. A qualitative phytochemical analysis was carried out and found to possess bioactive compounds like alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins.*

Keywords: *Achranthes aspera*, antimicrobial activity, aqueous extracts (hot and cold extraction), solvent extraction, rotary vacuum evaporator, Soxhlet, Cefuroxime.

I. INTRODUCTION

Free radicals are generated as part of the body's normal metabolic process, the free radical chain reactions are usually produced in the mitochondrial respiratory chain, atmospheric pollutants and from drugs, in biological systems, [1][2] In addition, chemical mobilization of fat stores under various conditions such as fever, infection, lactation, exercise, and even fasting can result in increased radical activity and damage [3][4]. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes [5]. Infectious diseases are the world's leading cause of premature deaths [6]. Thus, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Medicinal plants represent an enrich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [6][8]. In recent days the interest to evaluate plants possessing antibacterial activity for various diseases is rising [9] and it has also been proved that various plants extract poses bacteriostatic and bactericidal effects, and most of the plants contains many bioactive compounds [10]. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds [11]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals [6]. The medicinal actions of plants are unique to particular plant

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species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct. Therefore, it is worthwhile to use technology tools and modern science for verifying therapeutic potential of medicinal plants as antioxidant as per international standards. Such information may be of potential value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals-induced tissue damage [12]. From the above, the present study was carried out to evaluate antioxidant, antimicrobial activity and phytochemical analysis of *Achyranthes aspera*.

II. Materials And Methodology

2.1 Preparation of the Plant Extract

The freshly collected the plant parts of *Achranthes aspera* from the Anand Agriculture University. The plant parts were washed with distilled water and air dried at 40ºC by using hot air oven or dried at direct sunlight for 1 or 2 days. Then, make a powder by using grinder. The powder was collected in zip bag to prevent the moisture.

2.2 Plant Sample Extraction (Aqueous)

The plant sample was extracted by using aqueous method and solvent extraction method.

A. Hot Water Extraction

Take 5 to 10 gm of plant powder sample and add it to in 100 ml of distilled water in a conical flask and incubate to water bath at 80ºC for 2 to 3 hours. After that, the mixture was homogenized and filtered by using muslin cheese cloth and sample was proceed into rotary vacuum evaporator for evaporation of water and sample extraction. The temperature of rotary vaccum evaporator was depends on the boiling point of solvent to be used. After the evaporation, the sample was filtrated (Whatman filter paper) and collected in black screw cap bottle and stored in refrigerator.

B. Cold Water Extraction

Take 5 to 10 gm of the dried powder sample and added to 100 ml distilled water in a conical flak and incubate into orbital shaker incubator at 140 rpm for overnight. Next day, the mixture was homogenized and filtrated with use of muslin cheese cloth and proceed it in to rotary vacuum evaporator and after that the extract must filtrated with (Whatman filter paper) and the final extract collect in to the black screw cap bottle and stored in to refrigerator.

2.3 Plant Sample Extraction (Solvent)

Plant extract is extracted by using Soxhlet extractor with high efficiency to analyze the phytochemicals present in the extract and by using this extract we can perform the different assay. The temperature used in this method is based on the boiling point of solvent. The solvent used for the extraction of plant sample were Methanol (boiling temperature was 64.7ºC or 65ºC) and hexane (69ºC). Different solvent used because some phytochemicals are dissolved in polar and some are dissolved in non-polar solvent.

2.4 Phytochemical Analysis

- **Test for coumarins:** Take 2 ml of plant extract and add 10 % NaOH, formation of yellow color indicates the presence of coumarins.
- **Test for Anthocyanin:** Take 2 ml of extract and add 2N HCL and few drops of ammonia, if the anthocyanin is present in the sample the pink –red color turning to blue-violet color.
- **Test for steroids [Libermann Burchard Test]:** Take 1 ml of extract and add 10 ml chloroform and in that add equal amount of H_2SO_4 positive result gives upper layer red, while lower layer yellow with green fluorescence.
- **Test for saponins:** Take 2 ml of extract and add 6 ml of distilled water and then shaken it vigorously, foam was observed when the sample has saponins.
- **Test for terpenoids:** 2 ml of extract treated with 2 ml of acetic anhydride and then add few drops of H_2SO_4, positive result give blue, green ring formation.

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- **Test for tannins [Braymer's Test]:** Take 2 ml of extract and allowed it to react with 10% alcoholic ferric chloride solution, positive result gives the formation of blue, green color.
- **Test for phenolics:** Add Few drops of extract in to 5% aqueous ferric chloride and when this test is positive deep blue or dark color form.
- **Test for flavonoids [Alkaline reagent test]:** 2 ml of extract treated with 1N sodium hydroxide solution and give intense yellow color if, sample has flavonoids.
- **Test for alkaloids [Mayer's reagent]:** Add 2 ml of extract with few drops of Mayer's reagent and if, sample contain alkaloids than it will give white creamy precipitates.
- **Test for reducing sugar:** Take 0.5 ml of sample and add 5 ml of benedict reagent, boil it in boiling water bath for 1 min if the sample has reducing sugar, the solution form brick red color precipitates.

2.5 Test Microorganisms

The bacteria (*S. aureus, P. aeruginosa*)and fungi (*A. niger* and *Fusarium*) strain were collected from Shri Alpesh N Patel Post Graduation Institute of Science and Research, Anand.

2.6 Culture Media and Inoculums

The N-agar media used for the Anti-bacterial activity and PDA media was used for Antifungal activity. The bacterial culture was inoculated in the nutrient broth and incubated overnight to allow the growth.

2.7 Antibiotics

The antibiotic was used as a positive control in antimicrobial activity. In this activity cefuroxime was used as positive control and the concentration was 10mg/ml. In antifungal activity the Fluconazole was used, the concentration was 10 mg/ml.

2.8 Antimicrobial Screening

The antimicrobial activity of different parts extract carried out by agar well diffusion method.

A. Antibacterial Activity

The Antibacterial activity carried out by using agar well diffusion method and test microorganisms. The negative control of antibacterial activity are Methanol, Hexane and distilled water.

B. Antifungal Activity

The Antifungal activity was carried out by using well diffusion method. In this method the first step is growth of fungus on selective media, then make a suspension of it. If, fungi are sporulated, count the spores by hemocytometer and after that make a suspension which is used for the assay. Take an aliquot of 0.1 ml of suspension and spread it on the appropriate media, then make a well. Wells were filled with different parts extracts, in which for negative control the solvent and distilled water used and for positive control the Fluconazole was used.

Incubate the plates in incubator for 6 to 7 days at 25ºC to 30ºC. If the sample have an antifungal activity, the zone of inhibition was observed after the incubation time.

C. Antioxidant Activity

The antioxidant activity was determined by 2, 2 – diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging method. The anti-oxidant activity of different extract was measured in terms of H^+ donating or radical scavenging ability, using the stable radical DPPH. The different aliquots of extracts and 2 ml of DPPH in each tube was put in dark for 15 to 20 minutes and then take O.D at 517 nm.

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III. Results and Discussion

3.1 Phytochemical Analysis of Plant Extracts

The study of phytochemicals of *Achranthes aspera*shows that, Alkaloids, Flavonoids, Tannins, Saponins, Terpenoids, Steroids, reducing sugar, Coumarins, Phenolics are present [10] [13].

3.2 Antimicrobial Activity

A. Antibacterial Activity

The screening of antimicrobial activity of the *Achranthes aspera*shows the antibacterial activity against the *S. aureus, P. aeruginosa.*

B. Antifungal Activity

Antifungal activity was observed against *A. niger* and *fusarium* in *Achranthes aspera*, [13]. The extraction of *Achranthes aspera*gives the antifungal activity against *A. niger*, and some parts give antifungal activity against the *Fusarium*. The fluconazole antibiotic used as positive control, concentration is 10 mg/ml.

3.3 Antioxidant Activity

After screening out the antioxidant activity, the methanol extraction of *Achranthes aspera*gives high antioxidant activity [16] [17]. It also shows the absorbance of ascorbic acid increase with increasing of concentration of ascorbic acid. The radical scavage activity of ascorbic acid increase after 30 min of incubation in dark condition [14] [15].

3.4 Total Phenolics

Take 0.5 to 1 gm of plant sample and grind it with mortar and pestle in 10 times volume of ethanol.
Centrifuge the homogenate at 10,000 RPM for 20 min, save the supernatant. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (5 ml), pipette out different aliquots into test tubes. Make up the volume in each tube to 3 ml with water, add 0.5 ml of FCR reagent. After 3 min, add 2 ml of 20 % Na_2CO_3 solution to each tube, boil it in the boiling water bath and after that take O.D at 650.

IV. Conclusion

The present work demonstrates the antimicrobial potential of *Achranthes aspera* leaves and stems extract by using various solvents. The results indicate that n-hexane and methanol are better for the extraction of the antibacterial properties of *A. aspera*. The results also indicate that the plant extracts have antibacterial and antifungal effect, showing that they contain active ingredients against the organisms. The observed inhibition of bacteria and fungi, *Pseudomonas aeruginosa* and *Staphylococcus aureus and Fusarium* sp., and *Aspergillus niger*, respectively, suggests that *A. aspera*possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol or n-hexane as the solvent. Comparisons with related data from the literature indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained.

The study it provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of *A. aspera*. On the basis of the present finding, *A. aspera* leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by *P. aeruginosa* and *S. aureus*.

Phytochemicals shows the important part to prevent and protect the plant against the microorganisms. Another importance of phytochemicals of *A. aspera* provide the information about the compound which are responsible for antimicrobial activity like alkaloids, phenolics, flavonoids etc. The antioxidant activity was involved in the prevention of plant cell tissue damage. Antioxidant activity is measured by DPPH.

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V. TABLES, PICTURES AND GRAPHS

NB: '-' stand for negative whereas, '+' stand for positive.

Table 1: Phytochemical analysis of Aqueous extracts

Plant	Parts	extract	Coumarins	Steroids	Terpenoids	Flavonoids	Alkaloids	Saponins	Tannins	Phenolics	Reducing sugar
A. aspera	Leaves	Hot	-	-	+	+	-	+	+	+	+
		Cold	+	-	+	-	-	+	+	+	+
	Stems	Hot	+	-	+	+	-	+	+	-	+
		Cold	+	-	+	+	-	+	+	+	+

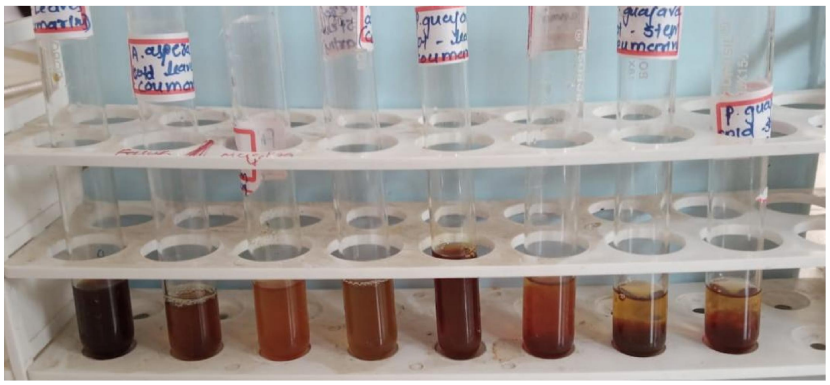

Image 1: coumarins test

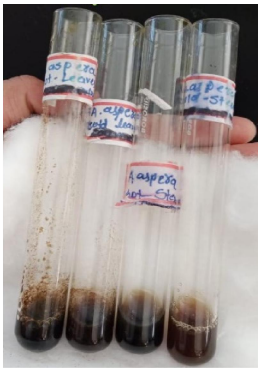

Image 2: Terpenoids

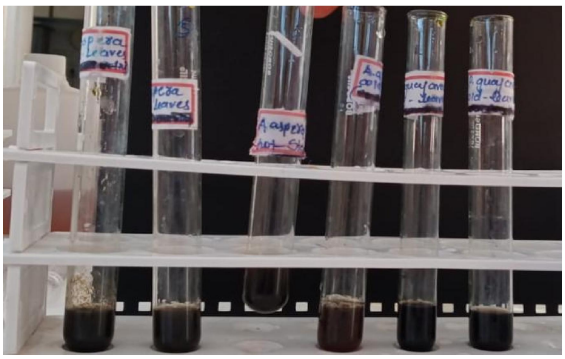

Image 3: Tannins

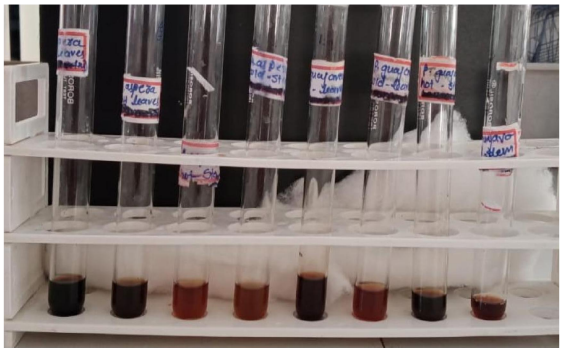

Image 4: Flavonoids

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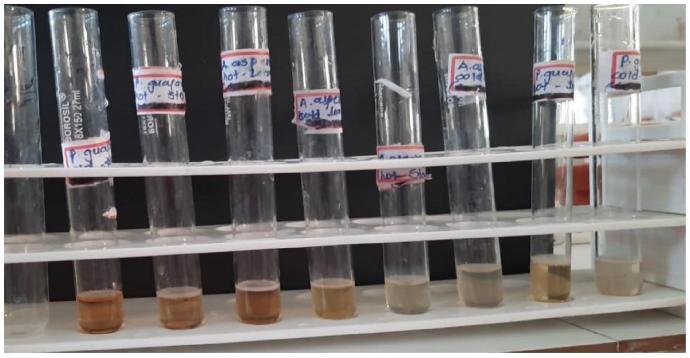

Image 5: Saponins

Table 2: Phytochemical analysis of Methanol extract

Plant	Parts	Coumarins	Steroids	Terpenoids	Flavonoids	Alkaloids	Saponins	Tannins	Phenolics	Reducing sugar	Gums	Glycosides	Carbohydrates
A. aspera	Leaves	-	+	-	-	+	-	+	-	-	-	-	-
	Stems	+	+	-	+	+	-	+	-	-	-	-	-
	Roots	+	-	-	+	-	-	-	-	+	+	+	+
	Seeds	+	-	+	+	-	-	-	-	-	-	-	-

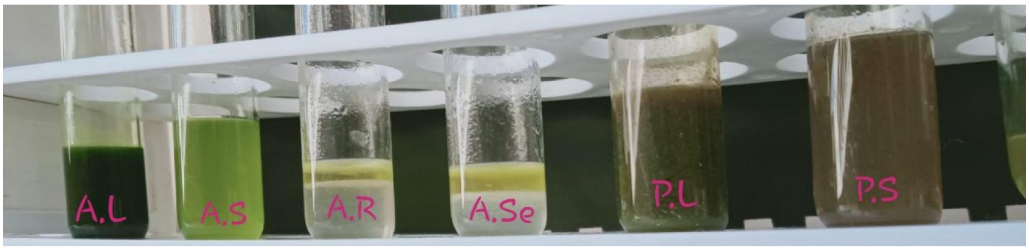

Image 8: Coumarins

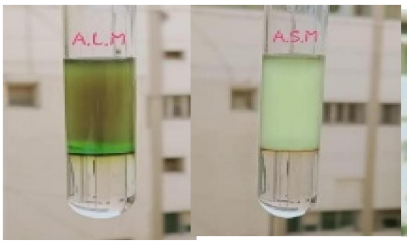

Image 9: Steroids

Image 10: Saponins

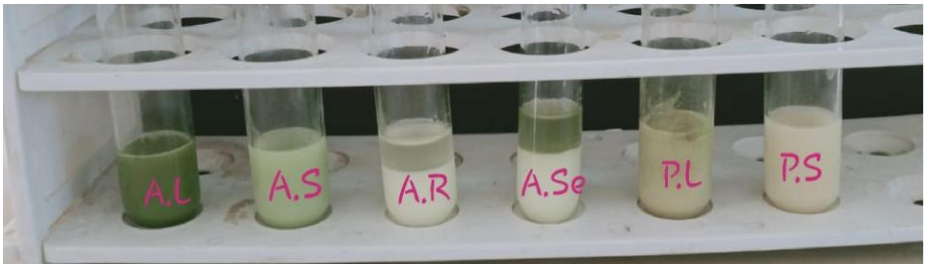

Image 11: Terpenoids

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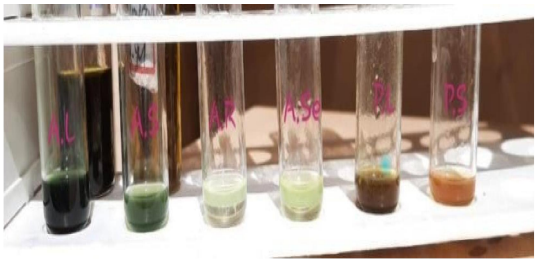

Image 12: Flavonoids

Image 13: Gums

Image 14: Alkaloids

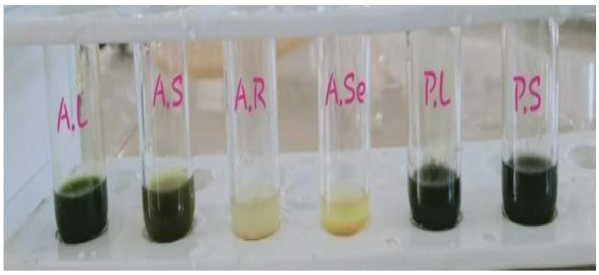

Image15: Tannins

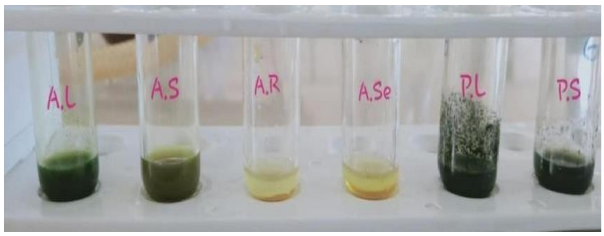

Image 16: Phenolics

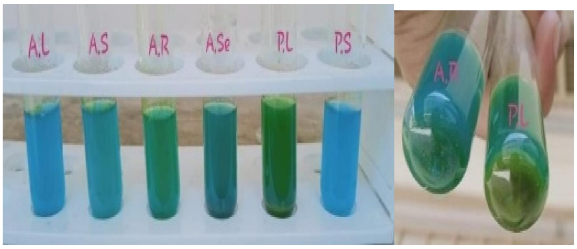

Image17: Reducing Sugar

Table 3: Phytochemical analysis of n- Hexane extracts:

Plants	Parts	Coumarins	Steroids	Terpenoids	Flavonoids	Alkaloids	Saponins	Tannins	Phenolics	Reducing sugar	Gums	Glycosides	Carbohydrates
A.aspera	Leaves	-	-	+	-	-	-	-	-	+	-	-	-
	Stems	-	-	+	-	-	-	-	-	+	-	-	-

Table 4: Antimicrobial activity of Aqueous extracts:

Plants	Parts	Extraction method	*S.aureus (mm)*	*P.aeruginosa (mm)*
A. aspera	Leaves	Hot	5	-
		Cold	-	-
	Stems	Hot	8	-
		Cold	9	3

Table 5: Antimicrobial activity of different extracts:

Plants	Parts	Solvent	*S.aureus* (mm)	*P.aeruginosa* (mm)
A. aspera	Leaves	Methanol	15	12
		N-hexane	18	17
	Stems	Methanol	13	10
		N-hexane	16	20
	Roots	Methanol	-	14
	Seeds	Methanol	15	13

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Image 18: *A. aspera*leaves and stems antibacterial activity against *Staphylococcusaureus*

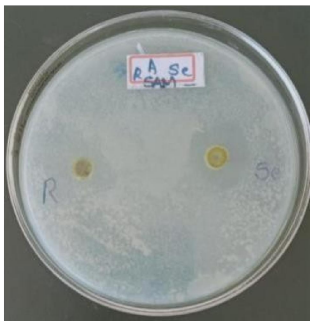

Image 19: *A. aspera*roots and seeds antibacterial activity against *Staphylococcusaureus*

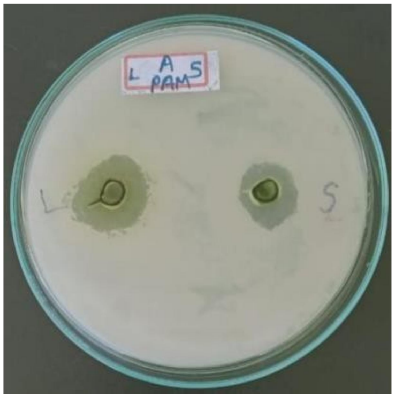

Image 20: *A. aspera*leaves andstems*a*ntibacterial activity against *Pseudomonas aeruginosa*

Image 21: *A. aspera* rootsand seeds antibacterial activity against *Pseudomonas aeruginosa*

Table 6: Antifungal activity against *A. niger:*

Plants	Parts	Solvent	*Aspergillusniger* (mm)
A. aspera	Leaves	Aqueous	9
		Methanol	3
		N-hexane	0
	Stems	Aqueous	5
		Methanol	6
		N-hexane	4
	Root	Methanol	0
	Seeds	Methanol	0

Table 7: Antifungal activity against *Fusarium* sp.:

Plants	Parts	Solvent	*Fusarium sp*. (mm)
A. aspera	Leaves	Aqueous (hot)	12
		Aqueous (cold)	12
		Methanol	0
		N-hexane	0

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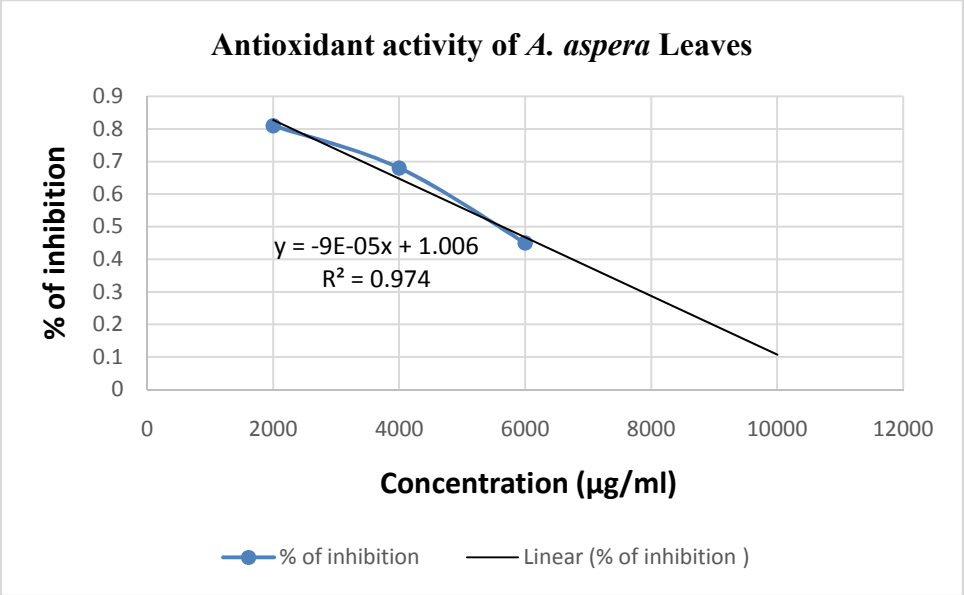
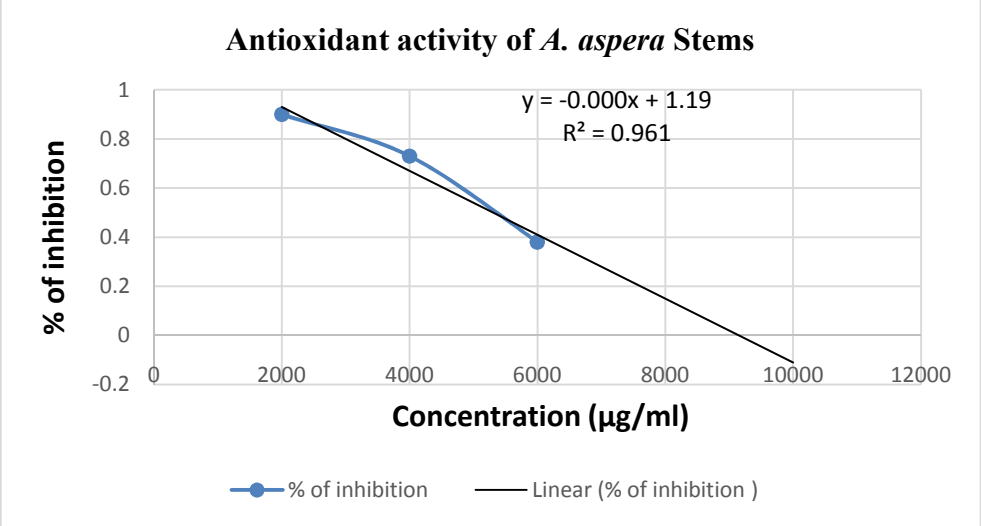
	Stems	Aqueous (hot)	0
		Aqueous (cold)	0
		Methanol	0
		N-hexane	0
	Roots	Methanol	0
	Seeds	Methanol	0

Graph 1: Antioxidant activity of *A. aspera* leaves



Graph 2: Antioxidant activity of *A. aspera* stems



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