

In vitro Antioxidant Potential and Cytotoxicity Study of *Asparagus aethiopicus* L. Extracts on HT-29 Human Colon Cancer Cell Line

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ABSTRACT

Objectives: To decide the phytochemical constituents, antioxidant and anticancer potential of *Asparagus aethiopicus* L. extracts on HT-29 human colon malignant growth cell line. **Methods:** The roots of plant were exposed to Hot Soxhlet continuous extraction with expanding polarity of solvents viz., pet ether, chloroform, ethanol and aqueous maceration. Qualitative phytochemical screening was completed by utilizing distinctive phytochemical tests. The antioxidant potential was tried utilizing 2, 2-diphenyl-1-picrylhydrazyl, ferric ion reducing power assay and phosphomolybdenum assay. *In vitro* anticancer action tried on HT-29 human colon malignant growth cell line and it was assessed by (3-(4, 5-dimethyl thiazole-2yl)- 2, 5-diphenyl tetrazolium bromide) MTT test. **Results and Discussion:** Preliminary qualitative phytochemical screening affirmed the presence of phytoconstituents like alkaloids, flavonoids, glycosides, saponins, sterols, tannins and reducing sugar. Antioxidant potential was demonstrated most noteworthy in ethanol extracts dependent on the test performed. The ethanol extracts were seen as an antioxidant and specifically cytotoxic to HT-29 human colon malignant growth cell line. **Conclusion:** The outcomes show that *Asparagus aethiopicus* L. having a potential of antioxidant activity and anticancer action on HT-29 human colon cancer cell line. In any case, further examinations are expected to presume that the particular constituent liable for its antioxidant action and cancer prevention agent.

Key words: Phytochemical, Antioxidant, Anticancer, Colon cancer, *Asparagus aethiopicus*.

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INTRODUCTION

Cancer is a disease described by uncontrolled engendering of cells that have changed from the typical cells of the body. The malignant growth cells can attack the neighbouring and distant tissues via the circulation. In advanced stages, a malignant growth patient may die because of either ill-advised finding or treatment disappointment. Malignancy is one of the push zones for which powerful medications at reasonable costs are not accessible until now presumably because of an absence of understanding the disease pathophysiology. For such a ghastly infection hostile to

malignancy drugs have been created from an assortment of sources extending from normal items (plants and organisms) to synthetic particles. The broadly utilized medications that are malignant growth chemotherapeutic specialists experience the ill effects of the downside of high danger, for example, bone marrow concealment, alopecia, queasiness and spewing and are not inside the compass of a typical man.¹ Medicines acquired from plants have assumed a central job in the social insurance of ahead of schedule and late societies. Ayurveda, the Indian arrangement of



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medication for the most part utilizes plant based medications or formulations to treat different sicknesses including malignancy. About 60% of medications allowed for cancer treatment are of natural source. Vincristine, Etoposide, Irinotecan, Taxanes and Camptothecines are instances of plant-derived anti-cancer compounds.²

A few malignancies inquire about investigations accompanied using traditional medicinal plants in solidarity to find new helpful specialists that do not have the dangerous reactions connected with current chemotherapeutic agents and the medications under clinical trials, phytomedicines have improved strongly in the previous two decades.³

Current treatment of malignant growth has significantly more side effects to defeat from that; natural remedies are helpful, adequate and demanding by the disease patients because of its less or no toxic effect. Henceforth here is a significant need to create substitute restorative techniques against this deadly illness. There is consistently the expectation that the pursuit among the traditional therapeutic plants may furnish strong and safe prescriptions with few or no poisonous impacts.

Reactive oxygen species are thought to assume a significant job in numerous human maladies. Radical scavenging power is essential because of the lethal job of free radicals in biological systems. Numerous secondary metabolites like phenols, polyphenols and flavonoids fill in as wellsprings of antioxidants and perform scavenging activity.⁴

In spite of assuming a key job in cell forms, reactive oxygen species promptly join and oxidize biomolecules, for example, sugars, proteins and lipids and in this manner making them inactive with sub-sequent harm to cells, tissues and organs prompting beginning of numerous maladies including malignancy. In this manner, by diminishing free radicals and oxidative pressure, antioxidants assume a job in improving DNA harm, decreasing the rate of abnormal cell division and lessening mutagenesis. Hence, numerous antioxidant-rich plants possess cancer prevention activity.^{5,6}

Asparagus aethiopicus L. (Sprenger plant/Asparagus greenery) has a place with the family Asparagaceae; Modern taxonomists have put the genus *Asparagus* in family Asparagaceae of request Asparagales rather than in Liliaceae. It is a helpful traditional medicinal plant, saw as developed all through the tropical and subtropical pieces of India. The dried roots of plants are generally utilized as a drug.⁷⁻¹¹ The plants from Asparagaceae family offers various medical advantages because of phytochemically a number of classes of phytoconstituents were isolated from plants of genus

Asparagus basically essential oils, flavonoids, saponin glycosides, tannins and sterols. The major bioactive constituents of asparagus are a gathering of steroidal saponins. This plant likewise contains vitamins A, B1, B2, C, E and Mg, P, Ca, Fe and folic acid. Other essential synthetic constituents of *Asparagus* are essential oils, asparagine, arginine, tyrosine, flavonoids (kaempferol, quercetin and rutin), gum, Shatavarin I–IV, asparagamine and tannin. From a pharmacological perspective, these varieties have antioxidant, cancer prevention, antidiabetic, anti-inflammatory, antimicrobial and immunostimulant exercises among others, affirming data got from ethno pharmacological studies.¹²⁻¹⁸ In any case, this plant has not been investigated for anticancer action on colon malignant growth and antioxidant properties of this plant. Subsequently, we have made an endeavor to utilize this natural plant to check the viability against colon malignant growth cell lines. The present investigation was intended to assess primer phytochemical assessment, *in vitro* antioxidant study and *in vitro* anticancer movement against HT-29 colon malignancy cell lines.

MATERIALS AND METHODS

Chemicals

All solvents were analytical grade. Pet Ether, Chloroform, ethanol, Ascorbic acid, ferric chloride, aluminium chloride, potassium acetate, DPPH reagent, sodium carbonate, were obtained from Loba Chemie Pvt. Ltd. Mumbai. Shimadzu 1800 UV-Vis Spectrophotometer was utilized in all spectrophotometric estimations.

Cell culture

The human colon cancer cell line (HT-29) was obtained from National Centre for Cell Sciences (NCCS), Pune, India. The cells were kept up at 37°C in a humidified atmosphere (90%) containing 5% CO₂ and afterword, cultured in (Dulbecco's Modified Eagle Medium) with low glucose, Thermo fisher scientific (Cat No-11965-092) with 10% (v/v) FBS, 100 units/mL penicillin and 100 µg/ml streptomycin.

Strategy for collection of plant

The *Asparagus aethiopicus* L. chosen for the investigation was collected from the Kolhapur district of Maharashtra. The plant was authenticated by Dr. S. R. Yadav, HOD and Taxonomist Department of Botany, Shivaji University Kolhapur and Herbarium deposited having Voucher specimen no. SSA-02 in the Department of Botany, Shivaji University, Kolhapur, Maharashtra, India.

Phytochemical evaluation

The root portion of *Asparagus aethiopicus* L. were collected, washed with water, shade dried and powdered. The powdered material was exposed to successive solvent extraction using Soxhlet apparatus with a selected order of polarity i.e. pet ether, chloroform, ethanol and water maceration. The extraction with solvent was continued until solvent from thimble of soxhlet apparatus get colourless. After extraction of every solvent, the powdered plant material was dried at room temperature and proceeds with next solvent extraction. The resulted successive extracts are concentrated on Rota evaporator and labelled as Pet ether Extract [PEAA], chloroform Extract [CEAA], Ethanol Extract [EEAA], Aqueous Extract [AEAA]. Then all extracts exposed to qualitative phytochemical investigation for the identification of phytoconstituents.

Tests for carbohydrates

The carbohydrates were tried by using Benedict's test, Fehling's test and Molisch test.

Test for proteins

Various extracts were dissolved in a few ml of water and treated with Million's reagent.

Tests for Fats and Oils

Fats and oils were tested with Translucent Spot test, Acrolein test,

Tests for sterols

The sterols were tested by using the Libermann-Burchard test and the Salkowski test.

Tests for glycosides

Keller Kiliani Test, Borntrager's test were utilized for the analysis of glycosides.

Test for saponins

Foam test was performed for the presence of saponins.

Test for Flavonoids

The flavonoids were tested by the Shinoda test and Ferric chloride test.

Tests for alkaloids

The alkaloids have been tested by using the Dragendroff's test and Wagner's test.

Tests for tannins

Test for tannins, ferric chloride test and lead acetate test were performed.¹⁹⁻²¹

Pharmacological Evaluation

In vitro antioxidant activity

The in-vitro antioxidant activity of different extracts of *Asparagus aethiopicus* L. studied by utilizing Ferric reducing antioxidant power (FRAP) assay, Phosphomolybdenum assay and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability assay.

Ferric ion reducing antioxidant power assay (FRAP)

Ferric reducing antioxidant power (FRAP) assay is a broadly utilized technique that utilizes antioxidants as reductants in a redox-connected colorimetric response, Ferric iron (Fe^{3+}) is initially reduced by electron-giving antioxidants present inside the sample to its ferrous form (Fe^{2+}). The iron colorimetric test complex builds a dark blue color product upon reduction which can be measured at 700 nm. Antioxidants are molecules that go about as reducing agents by giving electrons to free radicals to stabilize them and limit the harm brought about by free radicals to DNA, cells and organ systems. Antioxidant incorporates substances such as polyphenols; flavonoids; vitamins and enzymes like glutathione peroxidase and superoxide dismutase.²²

FRAP assay were utilized to quantify the total antioxidant potential of the extracts. Antioxidant activity assays were performed by the technique portrayed by Benzie and Strain with slight drugs. All extract of *Asparagus aethiopicus* L. and Ascorbic acid in various concentrations ranging from 12.5 $\mu\text{g}/\text{ml}$ to 200 $\mu\text{g}/\text{ml}$ and were blended with 2.5 ml of 0.2 mM phosphate buffer (pH 7.4) and 2.5 ml of potassium ferricyanide [1% weight/volume (W/V)]. The resulting mixture is incubated at 50°C for 20 min, followed by the addition of 2.5 mL of trichloroacetic acid (10% W/V) and centrifuged at 3000 rpm for 10 min. At that point, 2.5 ml of distilled water was included and later 0.5 ml of ferrous chloride (0.1% W/V). At long last, the absorbance was estimated at 700 nm. Ascorbic acid was utilized as a positive reference standard.²³

Phosphomolybdenum assay (PM)

The antioxidant assay depends on the reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V). The incubation of extracts with the Molybdenum (VI) will communicate the presence of antioxidant constituents in the extract, which can be assessed by recording the absorbance at 695 nm (to distinguish the reduced green molybdenum complex). In this way, this assay is very helpful to conceive the antioxidant capability of crude extracts.

The total antioxidant action was evaluated by Phosphomolybdenum (PM) assay utilizing the standard procedure of Prieto *et al.* All extracts of *Asparagus aethiopicus* L. and Ascorbic acid in different concentrations ranging from 12.5µg/ml to 200µg/ml were added to each test tube individually containing 3 ml of distilled water and 1 ml of molybdate reagent. These tubes were kept incubated at 95°C for an hour and a half. After incubation, they are kept at room temperature for 20–30 min and the absorbance was estimated at 695 nm. The positive reference standard was utilized in this assay was Ascorbic acid.^{23,24}

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability assay

The anti-oxidant capability of any compound can be resolved based on its scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. DPPH is a steady free radical containing an odd electron in its structure and generally used for the detection of the radical scavenging activity in chemical analysis.²⁵

The lambda max of a stable DPPH radical in methanol was at 517 nm. The decline in absorbance of DPPH radical is brought about by antioxidants, in view of the reaction between antioxidant particles and radical progresses, which brings about the scavenging of the radical by hydrogen donation.²⁶

Free radical scavenging effect of plant extract was resolved utilizing the 2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Briefly; the concentrations of extracts ranging from 12.5µg/ml to 400µg/ml were set up in ethanol. DPPH solution (0.004%) was prepared in ethanol and 1 ml of this solution was blended in with a similar volume of extracts and standard ascorbic acid solution separately. The mixture was incubated for half an hour in the dark at room temperature and the absorbance was measured at 517 nm. The level of DPPH purple decolorization to DPPH yellow showed the scavenging effectiveness of the extract. Lower absorbance of the reaction mixture showed higher free radical-scavenging activity. The scavenging activity against DPPH was determined utilizing the equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{AC}-\text{AT}}{\text{AC}} \times 100$$

AC: Absorbance of Control, AT: Absorbance of sample
The results were analyzed in triplicate. The IC₅₀ value is the concentration of the sample required to inhibit 50% of the DPPH free radical.^{23,27,28}

In-vitro anticancer activity

The viability assay most as often as possible utilized all through the world is the MTT assay. This colorimetric

assay utilizes a reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT) to measure cellular metabolic activity as a proxy for cell viability. Viable cells contain NAD(P) H- dependent oxidoreductase enzymes which reduce the MTT reagent to formazan, an insoluble crystalline product with a deep purple shading. Formazan crystals are then dissolved utilizing a solubilizing solution and absorbance is estimated at 500-600 nm utilizing a platereader. The darker the solution it indicates that more the quantity of viable and metabolically active cells.^{23,29-31}

The *in-vitro* cytotoxicity study of various extracts of *Asparagus aethiopicus* L. was carried out by utilizing HT-29 human colon malignant growth cell lines.

The cells were seeded at a density of around 5×10³cells/well in a 96-well flat-bottom microplate and kept at 37°C in 95% humidity and 5% CO₂ overnight. Distinctive concentration (800, 400, 200, 100, 50, 25 µg/ml) of samples were treated. The cells were incubated for extra 48 hr. The cells in well were washed twice with phosphate buffer solution and 20µl of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), staining solution (5mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37°C. After 4 hr, 100 µl of Dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals and the absorbance at 570 nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined. The % cell viability was determined utilizing the following formula:

$$\% \text{ cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100$$

A₅₇₀: Mean absorbance at 570nm^{23,32,33}

Statistical analysis

All the determinations of the experimental work were carried out in triplicates and the acquired information were classified and analyzed by using Microsoft Office Excel 2013. The results are expressed as mean values and standard Error Mean (SEM).

RESULTS

Qualitative Phytochemical evaluation

The existences of numerous secondary metabolites such as sterols, flavonoids, saponins, glycosides were recognized in ethanol and aqueous extracts. These significant metabolites were identified dependent on

shading changes, the formation of precipitation or formation of persistent forms qualitatively. On the other hand, a minor amount of carbohydrate, alkaloids and tannins were recognized. Traces of fats and oils were found in Pet ether extract. (Table 1)

In vitro antioxidant activity

Antioxidant capacities were shown highest in ethanol extracts based on the FRAP assay, PM assay and DPPH radical scavenging ability assay.

Ferric ion reducing antioxidant power assay

In the present investigation, extracts of *Asparagus aethiopicus* L. were exposed to FRAP assay alongside standard ascorbic acid. In the results obtained, Ethanol extract showed higher action than other extracts which was comparable to standard Ascorbic acid [AS]. (Figure 1)

Phosphomolybdenum assay

The Pet Ether (PEAA), Chloroform (CEAA), Ethanol (EEAA) and Aqueous (AEAA) extracts of *Asparagus aethiopicus* L. were exposed to PM assay along with standard ascorbic acid. In the results obtained, Ethanol extract showed higher activity than other extracts which was comparable to standard Ascorbic acid. (Figure 2)

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging ability assay

In the present study, Pet Ether (PEAA), Chloroform (CEAA), Ethanol (EEAA) and Aqueous (AEAA) extracts of *Asparagus aethiopicus* L. were subjected to DPPH free radical scavenging assay. The antioxidant potential of the extract was compared with ascorbic acid (AS) as the standard antioxidant. Ethanol extract showed higher activity than other extracts which was comparable to standard Ascorbic acid. (Figure 3) IC₅₀ values of DPPH scavenging activity of extracts

of *Asparagus aethiopicus* L. for antioxidant activity are represented in (Table 2, Figure 4).

In vitro anticancer activity

The result of MTT assays revealed that the ethanol extract was found to be selectively cytotoxic to HT-29 human colon cancer cell line as compared to other extracts compared with mostly used Paclitaxel as cytotoxic and normal control (NC). (Table 3, Figure 5). IC₅₀ values of extracts of *Asparagus aethiopicus* L. against HT-29 Colon cancer cell lines are represented in (Figure 6).

DISCUSSION

In recent years, the use of herbal medicines in cancer treatment has received increasing attention due to their varied Phyto-metabolic contents with multiple biological activities.³⁴ The plant collected from the Western Ghats was recognized by their taxonomical characters as *Asparagus aethiopicus* L. and analyzed for the presence of phytochemicals with four solvent extracts. Preliminary Qualitative phytochemical analysis revealed the presence of secondary metabolites in the selected extracts of the plant (Table 1). These secondary metabolites are reported to have many biological and therapeutic properties.³⁵ Among the various phytochemicals, sterols and flavonoids have picked up the regard for various zones of utilizations, for example, pharmaceutical, health, food and cosmetic ventures. These composites are basic in the plant space as piece of our everyday eating routine and are appealing as attractive natural antioxidants.^{36,37}

Reactive oxygen species (ROS) are thought to assume a significant job in numerous human illnesses. Free Radical scavenging activities are exceptionally vital because of the poisonous job of free radicals in biological systems.

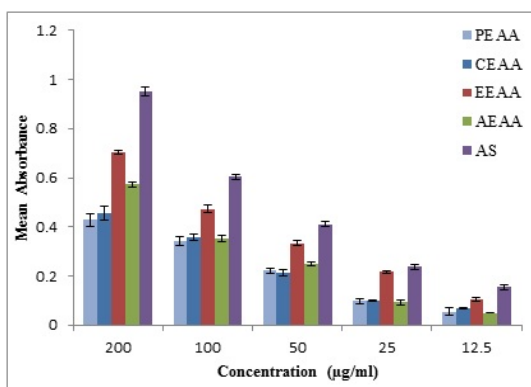


Figure 1: Mean absorbance at 700 nm of *Asparagus aethiopicus* L. extracts for FRAP assay. Data expressed as Mean \pm Standard error of the mean.

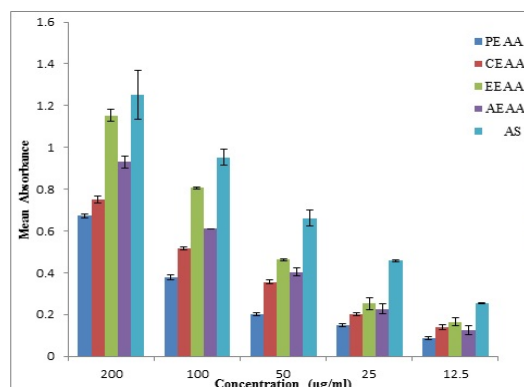


Figure 2: Mean absorbance at 695 nm of *Asparagus aethiopicus* L. extracts for PM antioxidant Assay. Data expressed as Mean \pm Standard error of the mean.

Numerous secondary metabolites like flavonoids fill in as origins of antioxidants and do scavenging activity.³⁸ ROS unreservedly consolidates and oxidizes biomolecules, for example, proteins, sugars and lipids in this manner making them lethargic with consequent harm to cells, tissues and organs prompting disease progression.^{39,40}

In the present work, FRAP, PM and DPPH strategies were utilized to assess the entire antioxidant capacity of pet ether, chloroform, ethanol and aqueous extracts.

The Ferric ion reducing capability of the extract may help as a significant presentation of its antioxidant activity. The presence of antioxidants, which have been shown to be an impart antioxidant action by breaking the free radical chain by donating a hydrogen molecule.⁴¹ The presence of antioxidants in the extract would bring about the reduction of ferricyanide Fe^{3+} to ferrocyanide Fe^{2+} by giving an electron which was estimated spectrophotometrically at 700 nm. In this assay, the yellow shade of the test solution changes to various shades of green and blue, depending on the reducing power of plant extract. The reducing

power decreased with the decrease in the extract concentrations. This might be filled in as a critical marker of its antioxidant potential. The ethanol extract indicated more absorbance at 700nm than other extracts (0.705 ± 0.007); subsequently this investigation assumed that ethanol extract of *Asparagus aethiopicus* L. may have a high measure of antioxidant property than other extracts which was comparable to that of the synthetic antioxidant standard used Ascorbic acid (Figure 1).

By utilizing phosphomolybdenum technique, the absolute antioxidant activity of the sample was investigated. It is a colorimetric quantitative technique that estimates the reduction of Phosphate-Mo (VI) to Phosphate-Mo (V) by the sample and resulting development of a pale blue green shaded Phosphate-Mo (V) complex.²³ It helps to watch the reduction rate among antioxidant and molybdenum ligand. In the present investigation, ethanol extract displayed higher absorbance at 695 nm than other extracts (1.153 ± 0.029) (Figure 2). Henceforth this examination perceived that ethanol extract of *Asparagus aethiopicus* L. may have a high measure of antioxidant property than other extracts

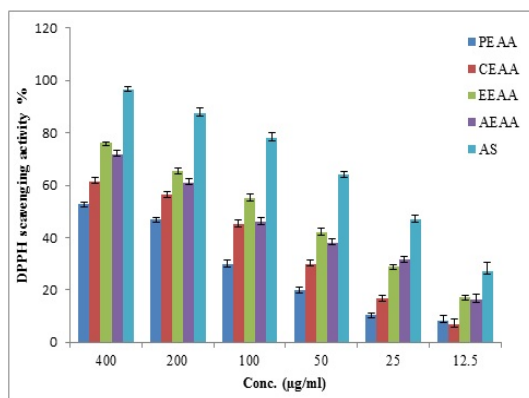


Figure 3: Percentage DPPH scavenging activity of *Asparagus aethiopicus* L. extracts for antioxidant activity. Data expressed as Mean \pm Standard error of the mean.

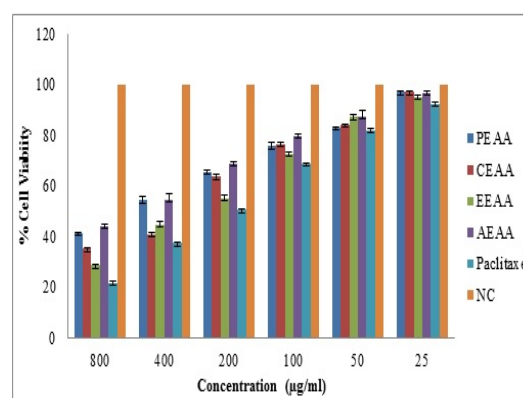


Figure 5: Percentage cell viability of *Asparagus aethiopicus* L. extracts for cytotoxicity on HT-29 colon cancer cell line. Data expressed as Mean \pm Standard error of the mean.

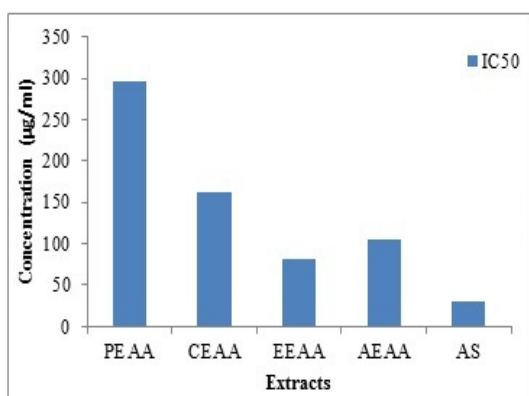


Figure 4: IC₅₀ values of *Asparagus aethiopicus* L. extracts for DPPH radical scavenging antioxidant activity.

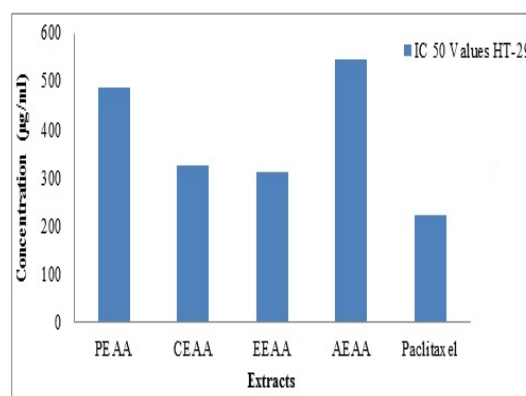


Figure 6: IC₅₀ values of *Asparagus aethiopicus* L. extracts for cytotoxicity on HT-29 colon cancer cell line.

Table 1: Qualitative Phytochemical Evaluation of *Asparagus aethiopicus* L. extracts.

Compounds	PEAA	CEAA	EEAA	AEAA
Carbohydrates	-	-	+	+
Proteins	-	-	-	+
Fats and Oil	+	-	-	-
Sterols	-	+	++	+
Glycosides	-	-	+	++
Saponins	-	-	+	++
Flavonoids	-	+	+++	++
Alkaloids	-	+	+	+
Tannins	-	-	+	+

+++ : Strongly present, ++ : Moderately present, + : Present in traces, - : Absent

Table 2: IC₅₀ values of extracts of *Asparagus aethiopicus* L. for DPPH antioxidant activity.

Compound	IC ₅₀ (µg/ml)
PEAA	296.7
CEAA	162.7
EEAA	82.26
AEAA	105.3
AS	29.64

which was practically comparable to that of synthetic antioxidant standard used Ascorbic acid.

DPPH is steady nitrogen focused free radical which is ordinarily used to decide free radical scavenging activities of antioxidants present in plant extracts or synthetic compound.⁴²⁻⁴⁴ The reduction capacity of DPPH radical is dictated by the decline in absorbance at 517 nm prompted by antioxidants. Greater absorbance

Table 3: Percentage cell viability of *Asparagus aethiopicus* L. extracts for cytotoxicity study on HT-29 colon cancer cell line.

Conc. µg/ml	PEAA	CEAA	EEAA	AEAA	Paclitaxel
800	41.44±0.25	35.24±0.58	28.65±0.33	44.21±0.57	21.87±0.68
400	54.41±1.51	41.03±0.54	44.68±1.36	54.76±2.31	37.39±0.43
200	65.59±0.62	63.68±1.10	55.24±1.13	68.91±0.76	50.53±0.50
100	75.74±1.42	76.62±0.82	72.79±0.69	79.85±0.99	68.83±0.51
50	83.09±0.30	84.27±0.02	87.35±1.07	87.65±2.32	82.01±0.84
25	97.18±0.70	97.21±0.33	95.24±0.58	97.06±0.59	92.6±0.48
NC	100				

Data expressed as Mean ± Standard error of the mean.

of the reaction mixture specified lesser free radical scavenging activity. In the present examination, ethanol extract displayed higher free radical scavenging activity than other extracts (76.33±1.09 %) (Figure 3). Thus this examination accepted that ethanol extract of *Asparagus aethiopicus* L. may have a high measure of antioxidant property than other extracts which was practically comparable to that of the standard synthetic antioxidant agent Ascorbic acid. The outcome indicated that ethanol extract significantly scavenges the free radical and was the most potent extract with an IC₅₀ value of 82.26µg/ml (Table 2, Figure 4).

The evaluation of the cytotoxicity of plant extracts is important for innocuous treatment. It empowers the identification of the inborn toxicity quality of the plant.^{45,46} The MTT assay is utilized in screening the crude extracts to assess the toxicity. It could likewise give a sign of conceivable cytotoxic properties of the tested plant extracts. MTT assay depends on the reduction of MTT by mitochondrial dehydrogenase by purple formazan product. It is often utilized as an *in vitro* model framework to measurement of cytotoxic impacts of plant extracts against malignant growth cell lines.^{47,48} *In vitro* cytotoxicity test using HT-29 colon malignant cell lines was performed to screen potentially

toxic compounds that affect basic cellular functions and morphology. The four extracts (Pet ether, Chloroform, Ethanol and Aqueous) of *Asparagus aethiopicus* L. appeared *in vitro* development hindrance consequences for the malignant growth cell lines (HT-29), while there was no impact on the development of normal cells. Such specific impacts were focus just as, incubation period subordinate, regarding concentration (25, 50, 100, 200, 400, 800 µg/ml) of each extract were assessed in triplicates by sequential dilutions. Among these six concentrations, 800 µg/ml of ethanol extract was the best in creating percentage growth inhibition (Cell viability: 28.65±0.33 %) trailed by chloroform extract (Cell viability: 35.24±0.58 %) when compared with other extracts. However, the standard paclitaxel drug showed significant inhibition (Cell viability: 21.87±0.68 %) on the malignant growth cell lines (Table 3, Figure 5). The outcome indicated that ethanol extract is having potential of anticancer activity with an IC₅₀ value of 313.2µg/ml (Figure 6).

CONCLUSION

It was seen that the plant *Asparagus aethiopicus* L. contains a wide assortment of secondary metabolites that hold potential to be a source of antioxidants and anticancer capability dependent on the analyses performed, which gives a logical proof to direct further examinations and research the lead compounds present in the plant and assess its anticancer potential on other cancer cell lines and on *in vivo* animal models and set forward an endeavour to carry out trials on human beings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl; **FRAP:** Ferric reducing antioxidant power; **PM:** Phosphomolybdenum assay; **MTT:** (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **PEAA:** Pet ether Extract

of *Asparagus aethiopicus* L.; **CEAA:** Chloroform Extract of *Asparagus aethiopicus* L.; **EEAA:** Ethanol Extract of *Asparagus aethiopicus* L.; **AEAA:** Aqueous Extract of *Asparagus aethiopicus* L.; **AS:** Ascorbic acid; **NC:** Normal control.

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About Authors

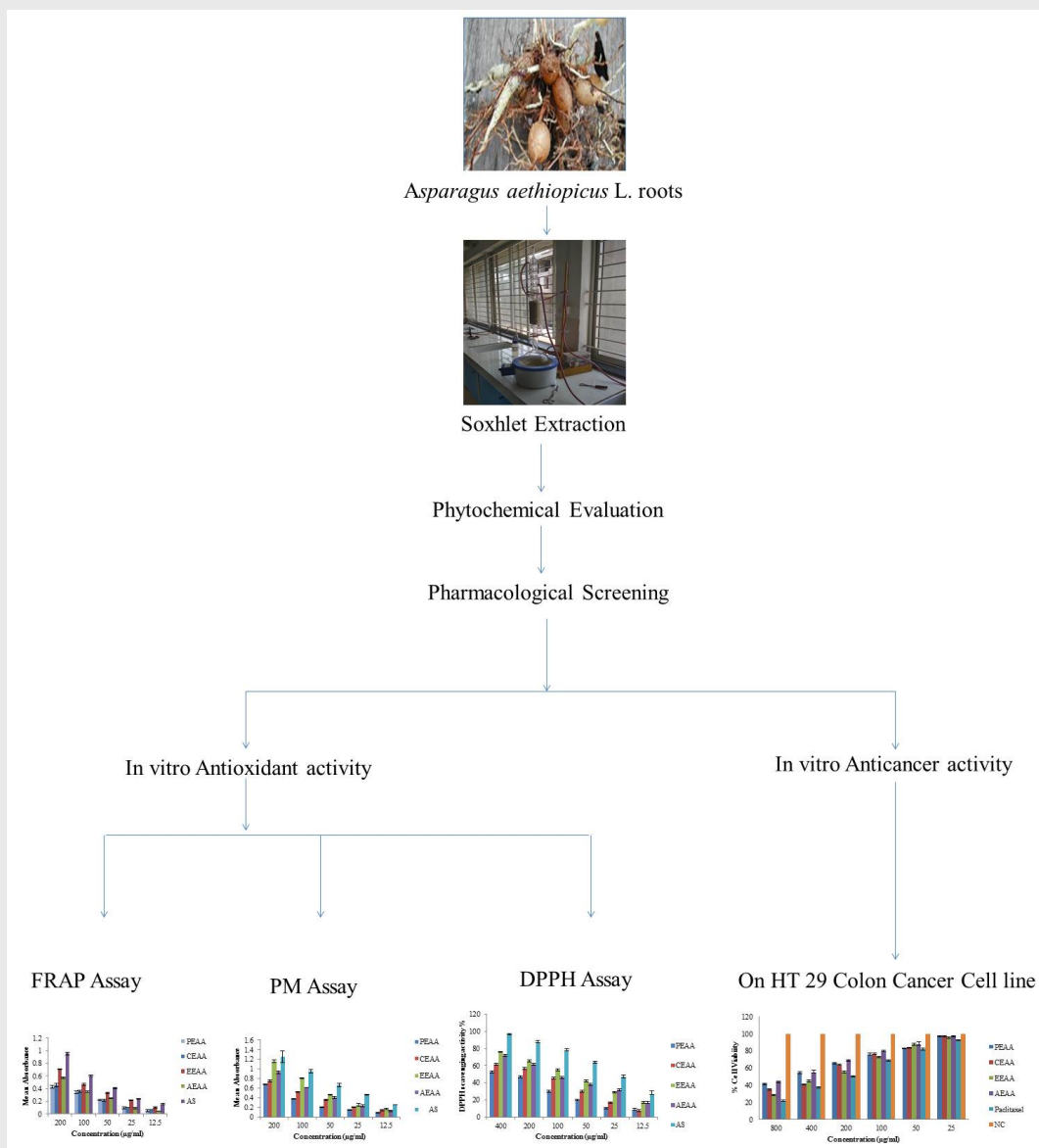


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SUMMARY

- *Asparagus aethiopicus* L. contains a wide assortment of secondary metabolites like flavonoids, sterols, glycosides, alkaloids etc.
- These secondary metabolites hold potential to be a source of antioxidants and anticancer capability dependent on the analyses performed.

PICTORIAL ABSTRACT



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