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Phytochemical, elemental, physico-chemical, HPTLC and anticancer investigations of *Ceropegia spiralis* Wight. Tuber extracts

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Abstract
Phytochemical constituents are responsible for medicinal activity of plant species. Hence in the present study Qualitative and quantitative phytochemical screening, Physicochemical, elemental analysis and anticancer activity of *Ceropegia spiralis* tuber extracts were carried out. Qualitative and quantitative phytochemical analysis of tuber aqueous extract confirm the presence of various secondary metabolites like saponins, triterpenoids, steroids, tannins, alkaloids, flavonoids and phenols. The results suggest that the phytochemical properties for curing various ailments and possess potential anticancer, antimicrobial and antioxidant activities leads to the isolation of new and novel compounds. Physicochemical studies reveals that the dry matter 96.23%; followed by Water soluble extractive 14.28 %. Elemental analysis reveals Nitrogen 3.4 % followed by Zinc 218.9%. Along with this, macro and micro elements which are essential for maintaining the animal body were also determined quantitatively. Anticancer activity of *C. spiralis* exhibited potential towards MDAMB-231 (human breast cancer) cell lines, shows 78.30% cell death with cell viability 21.70% at 100 µg/ml. The presence of various bioactive compounds confirms the application of *C. spiralis* against many ailments by the traditional practitioners.

Keywords: *Ceropegia spiralis* - quantitative, steroids, alkaloids, flavonoids, phenols, MDA MB-231

Introduction
Medicinal plants are the richest bio-resources of folk medicine traditional systems of medicine; food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs [1]. Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening [2]. India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurveda and Unani. Traditional systems of medicines are prepared from a single plant or combinations of many plants. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in the raw drug [3]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [4]. Screening of active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment against various diseases, including cancer [5] and Alzheimer diseases [6].
Phytochemicals are basically divided into two groups that is primary and secondary metabolites based on the function in plant metabolism. Primary metabolites are comprise common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on [7-8]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug [9]. There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease [10].
The Genus *Ceropegia* was earlier included in the family Asclepiadaceae has been reduced to sub family Asclepiadiodeae under the family Apocynaceae. There are nearly 710 species of the tribe Ceropegieae (Apocynaceae Asclepiadiodeae) [11&12]. The genus *Ceropegia* L. (1753:211), was the largest in the tribe Ceropegieae represented by 244 taxa worldwide, distributed only in the old world ranging from the Spanish Canary Islands in the west, through

central, Southern and Northern Africa, Madagascar, Arabia, India, Southeastern Asia and South Western pacific region [13].

The selected medicinal plant *C. spiralis* Wight (Apocynaceae) is a slender, erect herb with depressed tubers, opposite leaves, sessile 10-20cm long narrowly linear, base and apex often curved and twisted at the tip. Flowers 3-5 cm long. Greenish-purple, on cymes, mostly solitary. Fruit of two slender follicular mericarp (Fig 1). [14]. Flowers peculiar with ornamental potential. It is endemic to Peninsular India [15].

The tuberous roots are edible which contain starch, sugar, gum, albuminoids, fats, and crude fibers are valuable constituents in many traditional medicinal systems of India [16]. *Ceropegia* species are storehouse of various valuable phytoconstituents that are routinely used in traditional Indian ayurvedic drugs for the treatment of gastric disorders, diarrhoea, dysentery, urinary tract ailments dysentery, and to cure sneezing, cold and eye diseases in Bihar region and also the seed paste has been used for treatment of Deafness, etc [17]. Pharmacological importance of the genus *Ceropegia* is mainly due to the presence of pyridine alkaloid "cerpegin", which is potentially antipyretic, analgesic, local anesthetic, antiulcer, mast cell stabilizing, hepato-protective, tranquilizing, and hypotensive [18]. Poor seed setting, low seed germination, scarcity of pollinators and indiscriminate exploitation of edible tubers of *C. spiralis* seems to be the main hindrance for its natural regeneration to maintain the wild population. The genus, *Ceropegia* is under threat owing to either destructive collection or habitat degradation. Fifty species are present in India [19]. Out of which 28 species are endemic to Peninsular India [20, 21].

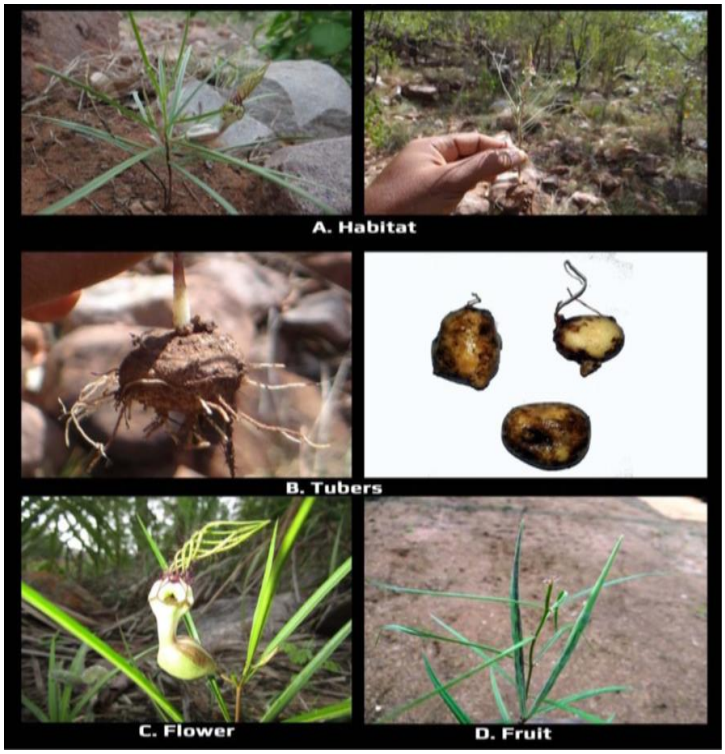


Fig 1: *Ceropegia spiralis*

Material and Methods

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of different extracts of tuber were carried out according to Standard methods [22-24].

Quantitative Phytochemical Analysis

Determination of Total Tannin, phenolic and flavonoid contents were carried out by the following methods [25-30].

Elemental analysis: Elemental analysis of the aqueous tuber extract was done by the standard procedures [31].

Physico Chemical Analysis: Physico-Chemical analysis of the selected plant material was done by the standard procedures [31-34].

Statistical analysis

All the Experiments were conducted in triplicate and results were expressed as mean ± standard error. Statistical analyse was done by one-way ANOVA followed by Dunnett's test with $P < 0.05$ as a limit of significance.

Anticancer activity

Human breast cancer (MDA-MB-231) cell lines were procured from National Centre for Cell Science, Pune, India. The 0.2 ml of Dulbeccos Modified Eagles Medium was used to growing up 1×10^4 cells per well in 96-wells plate. These cell lines were incubated in 5% CO_2 atmosphere at 37^0C for 24 h supplemented with 2 mM/l glutamine, 10% Foetal Bovine Serum (FBS) with 10 µg/ml of ciprofloxacin as control [35]. After that medium was expelled and refilled with 0.15 ml of 10, 25, 50 and 100 µg/ml concentrations of *C. spiralis* tuber Aqueous extract. The 0.1% of DMSO (dimethyl sulfoxide) was prepared for dissolving sample nanoparticles as well as MTT dye crystals has been set as negative control and 1 µM doxorubicin treated cell lines were set as positive control. The initial experiment was maintained from 0 to 72 h of timeline period with 12 h of time gap period to check probability of cell toxicity. It provides specific time course period to allow functional cell mortality to understand the experiment in a flexible and adaptable way. According to that, less cell toxicity was observed at 12-, 24-, 36-h period and greater cell toxicity was observed at 60 and 72 h period. It

reduces the chance of taking readings with ELISA reader. The 48 h of time period showed optimum reliability than other timeline periods. Due to this, the 48 h of incubation period was considered for nanoparticles cytotoxicity prediction analysis. Triplicates of experiments were carried out and incubated them up to 48 h at 37^{0}C.

Further, at the end of incubation period the sample solutions were discarded and incubated for 4 h at 37 ^{0}C by adding 0.02 ml of MTT reagent (5 mg/ml) to each well. After that, MTT containing medium was discarded and refilled with 0.15 ml/well DMSO (dimethyl sulfoxide) to dissolve formazan crystals. The viability of cell lines was read at 570 nm by an ELISA reader. The percentage of cell viability was calculated by the following formula [36].

$$\text{Percentage of Cell viability} = \frac{\text{OD value of treated cell lines}}{\text{OD value of control}} \text{ X } 100$$

High-Performance Thin Layer Chromatography (HPTLC) analysis

Thin-layer chromatography and HPTLC is one of the important separation chromatographic techniques used for detecting the adulteration for assessing the quality of the drugs through fingerprint profile of the drug. If the drug is adulterated there might be the appearance of the other compounds, in turn may increase the no of spots. On the other hand, the exhausted or deteriorated drugs may lose the component, and the number of spots appeared might be less. High-performance thin-layer chromatography (HPTLC) is a popular method for quality control of herbal products and the analysis of herbal medicines. It is widely used for separation, qualitative and quantitative estimation of marker compounds present in herbal drugs. HPTLC fingerprint profile is suitable for standardization of components followed by determination of specific bio-active phytoconstituents from plant materials. The HPTLC fingerprint for the formulation was developed. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of sample [37-38].

Preparation of alcoholic extract of the SM for HPTLC analysis

Five gram of powdered sample is taken and reflux with 200 ml of alcohol using a soxhlet apparatus on a water bath for 30 minutes. Filter the extract and concentrate to 5 ml then the sample extract obtained so far is used for further analysis.

HPTLC method conditions

The sample extract was spotted on pre-coated aluminum sheets of silica gel 60 F254 (Merck) with the help of automatic TLC applicator system of the DESAGA Sarstedt Gruppe. After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated in its selected proportional ratio and developed in the twin through chamber of TLC to the 80mm height of the plate to separate the components on the polar phase of silica gel and that of the mobile phase of the solvent system.

Development of HPTLC technique

After developing, TLC plate was air-dried and detected with the suitable detection system like UV Cabinet system for detection of spots at 366nm, 254nm and also under iodine vapours and after derivatizing with anisaldehyde sulfuric acid reagent as shown in the figure 1. Further, it was scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 366nm, 256nm, under exposure to iodine vapours at 580nm and after derivatization with anisaldehyde sulfuric acid reagent at 580nm. The typical densitograms obtained upon scanning under densitometer under the specific conditions for the above detection system were shown, which peaks appeared for the corresponding spots being detected in the densitometer. The peak areas in the densitogram correspond to the concentration of the component in the sample. The suitable separation of the components was developed for the important formulation, and the Rf values were recorded.

Results

Physicochemical Analysis of *C. Spiralis*

(Table – 1; Figure - 2) tuber dry matter 96.23%, water soluble extractive 14.28%, crude protein 7.67%, total ash 7.52% and cellulose 7.15%; alcohol soluble extractive 6.54%, loss on drying 5.32%, lignin 1.5%, acid insoluble ash 0.79% and in hemicellulos 0.21%:

Table 1: Physicochemical Analysis of *C. Spiralis*

S. No	Parameter	CS
1	Cellulose %	7.15
2	Hemicelluloses %	0.21
3	Lignin %	1.5
4	Dry matter %	96.23
5	Crude protein %	7.67
6	Loss on Drying at 105^{O}C	5.32
7	Water soluble extractive	14.28
8	Alcohol soluble extractive	6.54
9	Acid insoluble ash	0.79
10	Total ash	7.52

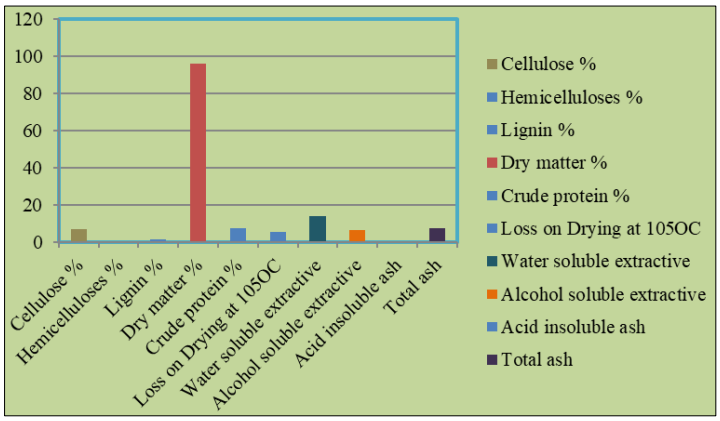


Fig 2: Graphical representation of physicochemical analysis of *C. spiralis.*

Elemental (ICP - OES) analysis of *C. spiralis:* (Table - 2)
The results of elemental analysis (Table – 2) of the tuber samples shows that *C.spiralis* exhibits the highest concentration of Iron (Fe) 1740 ppm, Zinc (Zn) 218.9 ppm, Boron (B) 93.08 ppm, Manganese (Mn) 5.11 ppm, Copper (Cu) 65.07 ppm, Molybdenum (Mo) 42.79 ppm and the very lowest concentration of Nitrogen (N) 3.4%, Potassium (K_2O) 2.20%, Calcium (Ca) 1.47%, Phosphorous (P_2O_5) 0.48% and Magnesium (Mg) 0.43%.

Table 2: Elemental (ICP - OES) analysis of *C. spiralis*

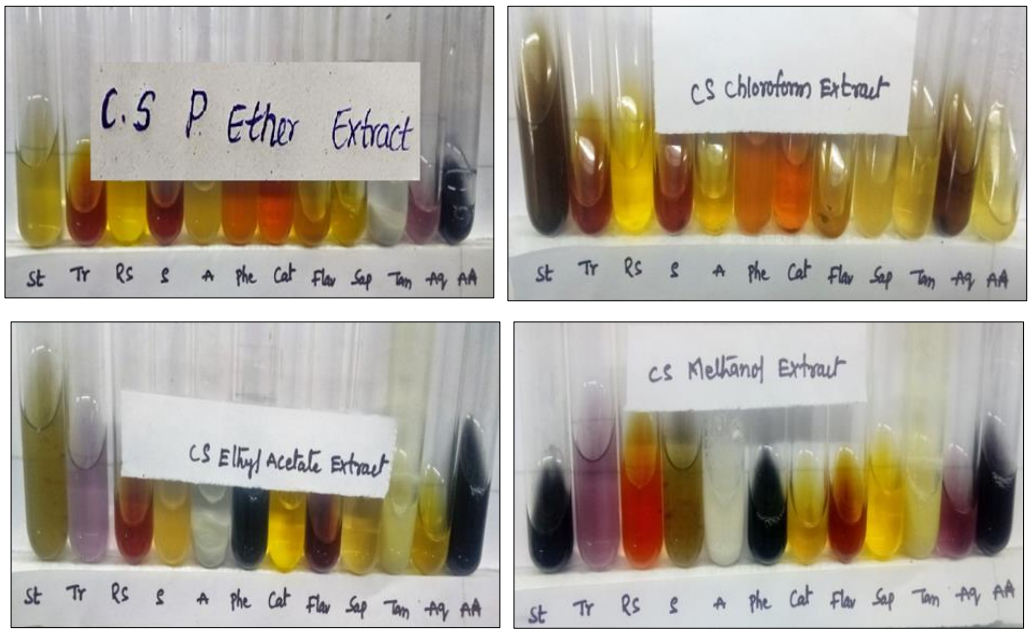
Parameters	Unit	Reading
Lab reference		24
Your reference		CS
Nitrogen (N)	%	3.4
Phosphorus (P_2O_5)	%	0.48
Potassium (K_2O)	%	2.20
Calcium (Ca)	%	1.47
Magnesium (Mg)	%	0.43
Zinc (Zn)	ppm	218.9
Iron (Fe)	ppm	1740
Copper (Cu)	ppm	65.07
Manganese (Mn)	ppm	65.11
Boron (B)	ppm	93.08
Molybdenum (Mo)	ppm	42.79

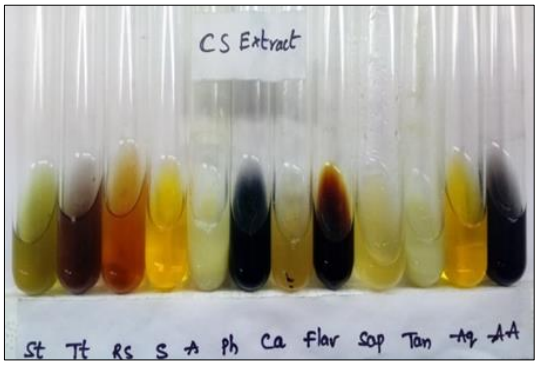
Qualitative analysis of *C. spiralis*: (Table - 3; Fig 3)
Qualitative analysis of tuber extracts reveals the presence of eleven compound include saponins, tannins, amino acids, steroids, triterpenoids, reducing sugars, alkaloids, phenols, flavonoids, sugars, phenols and anthroquinones. Tuber yielded a good number of compounds in ethyl acetate, methanol and aqueous extracts. Where as in the benzene and chloroform extracts only steroids, saponins, tannins and amino acids are present.

Table 3: Qualitative Analysis of *C. spiralis*

S. No.	Test	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
1	Steroids	-	+	-	+	-
2	Tri terpenoids	-	-	+	+	+
3	Reducing Sugars	-	-	+	+	+
4	Sugars	-	-	-	+	-
5	Alkaloids	-	-	+	+	+
6	Phenols	-	-	+	+	+
7	Catechins	-	-	-	-	-
8	Flavonoids	-	-	+	+	+
9	Saponins	+	+	+	+	+
10	Tannins	+	+	+	+	+
11	Anthroquinones	-	-	-	+	-
12	Amino Acids	+	-	+	+	+
Total		3	3	8	11	8

(+ Present, - absent)





(St: Steroids Tr: Triterpenoids Rs: Reducing sugar S: Sugars A: Alkaloids, Phe: Phenolic compounds, Cat: Catechins, Fla: Flavonoids, Sap: Saponins, Tan: Tannins, Aq: Anthroquinones and AA: Amino acids).

Fig 3: Phytochemical analysis *C. spiralis*

Quantitative analysis

Total Flavonoid content of *C. spiralis*: (Table-4; Figure-4)

The total flavonoid content (TFC) of *C. spiralis* ranged from 51.25±3.30 to 106.±3.81QE mg/g. The results revealed a considerable diversity in the TFC among the various extracts observed in the present study. The highest was found in methanolic tuber extract 106.66±3.81 QE mg/g and the lowest was found in petroleum ether 51.25±3.30 QE mg/g.

Table 4: Total Flavonoid content of *C. spiralis*

Extraction	Total flavonoid content (QE mg/g)
Petroleum ether	51.25 ± 3.30
Chloroform	55.83 ± 1.909
Ethyl acetate	77.91 ± 2.602
Methanol	106.66 ± 3.81
Aqueous	63.33 ± 1.909

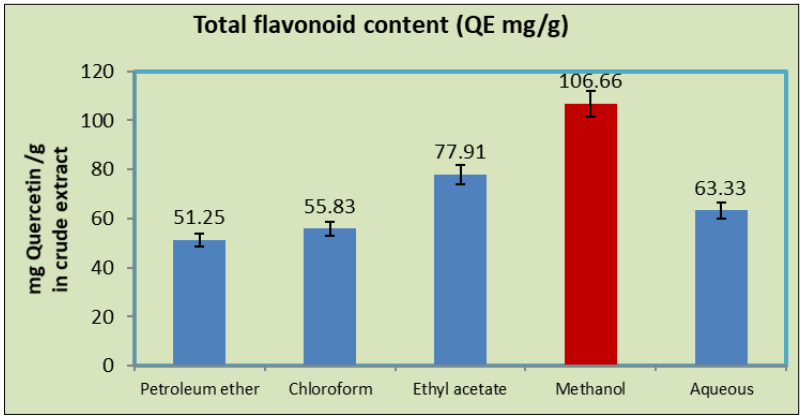


Fig 4: Total Flavonoid content of *C. spiralis*

Total Phenolic content of *C. spiralis*: (Table - 5; Figure - 5)

The quantitative analysis of phenols (TPC) in different extracts varied widely ranging from 28.33±2.51 to 48.66±3.51 GAE mg/g. The results revealed that highest level of phenols was observed in methanol tuber extract i.e., 48.66±3.51 QAE mg/g and the lowest level of Phenols was observed in 28.33±2.51 QAE mg/g.

Table 5: Total Phenolic content of *C. spiralis*

Sample	Total Phenolic content GAE mg/g
Petroleum ether	33 ± 1
Chloroform	28.333 ± 2.516
Ethyl acetate	42.33 ± 1.52
Methanol	48.66 ± 3.511
Aqueous	34.66 ± 2.516

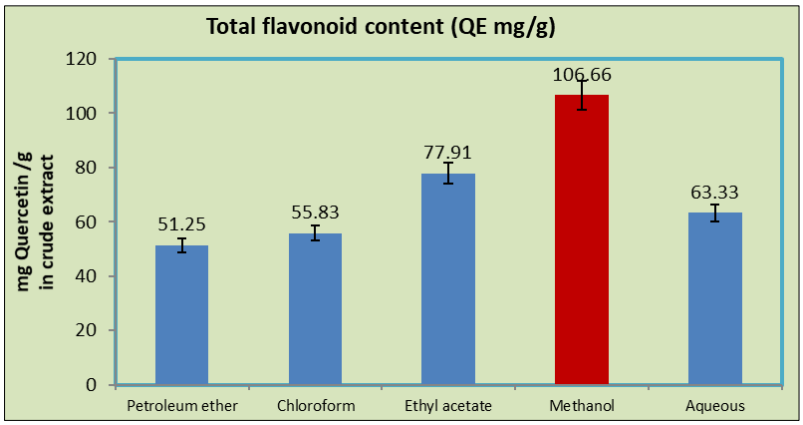


Fig 5: Total Phenolic content of *C. spiralis*

Total Tannin content of *C. spiralis* (Table – 6; Figure - 6)

The tannin contents of tuber extract of *C. spiralis* was found ranging from 44.58 ± 3.44 GAE mg/g to 114.58 ± 4.38 GAE mg/g of dry sample. The highest TTC was found in methanolic extract 114.58 ± 4.38 GAE mg/g and the lowest TTC was found in petroleum ether 44.58 ± 3.44 mg GAE mg/g.

Table 6: Total Tannin content of *C. spiralis*

Sample	mg GAE/g
Petroleum ether	44.583 ± 3.442
Chloroform	49.583 ± 5.051
Ethyl acetate	79.166 ± 1.909
Methanol	114.583 ± 4.389
Aqueous	107.708 ± 1.572

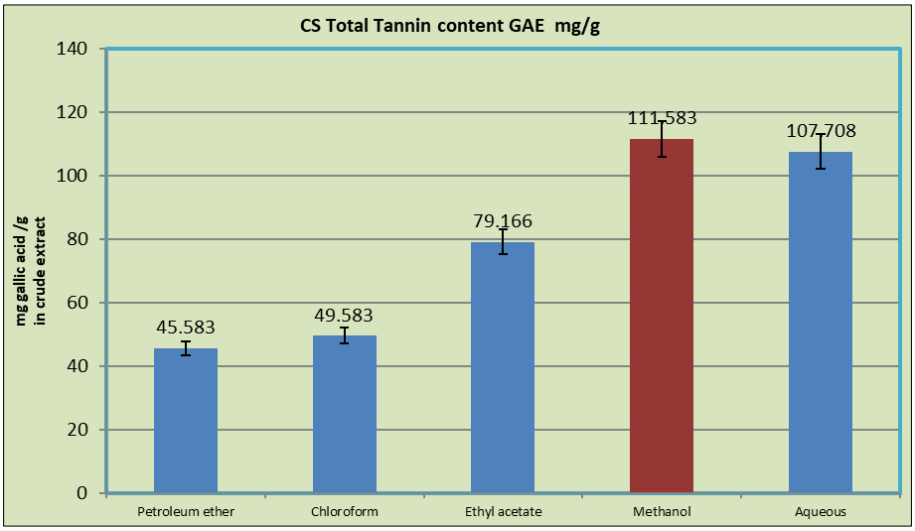


Fig 6: Total Tannin content of *C. spiralis*

HPTLC analysis of an alcoholic extract of *C.spiralis*

The alcoholic extract of ***C.spiralis*** 10 µl was spotted on silica gel "G" plate using applicator and developed in twin through a chamber with toluene: ethyl Acetate (7:3 v/v) as mobile phase. The air-dried TLC plate shows eight major spots under UV 366nm at R_f values (fig.7a & Table7) 0.04 (blue), 0.07 (red), 0.21 (blue), 0.28 (blue) 0.4 (red), 0.47 (blue), 0.64 (blue), 0.68 (blue) and under UV 254nm shows four spots at R_f values 0.24, 0.47, 0.64, 0.71 (All black); and under Iodine vapours shows (fig.7b & Table8) two spots at R_f values 0.2, 0.64 (brown) as shown in table 1 to 3 and Densitogram representation shown in figure 1 to 3 respectively.

The TLC studies of alcoholic extract of *C.spiralis* was performed for the separation of different compounds present in the solvent extract, and R_f values of various spots appeared in the TLC plate were calculated respectively. The TLC of alcoholic extract of *C.spiralis* with mobile phase solvent system as toluene: ethyl acetate (7:3v/v) was developed and detected in various detection system such as UV 366nm, 254nm and exposure to iodine vapours is studied. The Rf values corresponding to each spots was observed and recorded as eight major spots under UV 366nm at R_f values 0.04 (blue), 0.07 (red), 0.21 (blue), 0.28 (blue) 0.4 (red), 0.47 (blue), 0.64 (blue), 0.68 (blue) and under UV 254nm shows (fig.7c & Table9) four spots at R_f values 0.24, 0.47, 0.64, 0.71 (All black); and under Iodine vapours shows two spots at R_f values 0.2, 0.64 (brown).

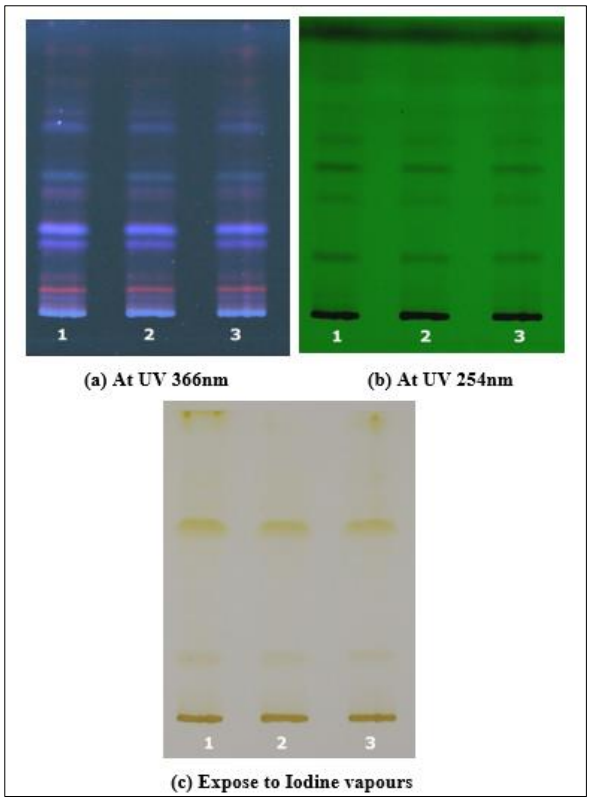


Fig 7: TLC of Alcoholic extract of *C. spiralis*

HPTLC of Alcoholic extract of *C. spiralis*

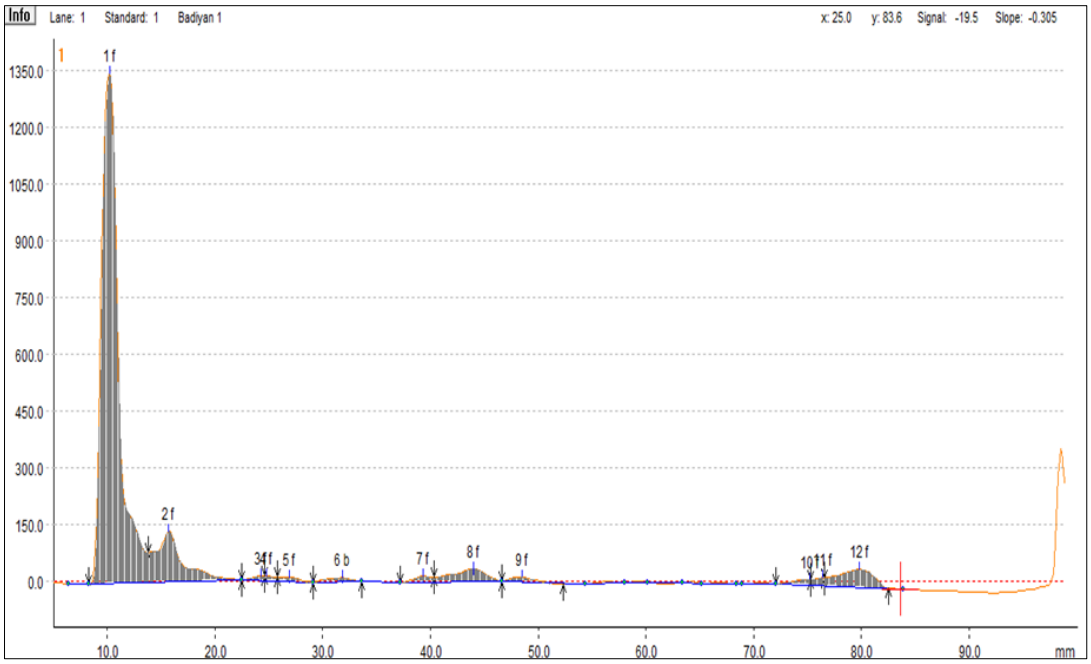


Fig 7a: Densitogram of Alcoholic extract of *C. spiralis* at UV 366nm

Table 7: Peak list of Alcoholic extract of *C. spiralis* at UV 366nm

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	10.2	2479.36	73.82	1339.15	0.02
2	15.7	389.67	11.60	133.80	0.09
3	24.3	15.02	0.45	11.95	0.21
4	24.8	12.69	0.38	12.30	0.22
5	26.9	20.08	0.60	10.16	0.25
6	31.8	24.96	0.74	10.16	0.32
7	39.3	28.32	0.84	15.91	0.42
8	44.0	121.57	3.62	33.94	0.49
9	48.5	29.01	0.86	12.37	0.55
10	75.2	27.24	0.81	15.28	0.92
11	76.4	24.32	0.72	21.47	0.94
12	79.8	186.39	5.55	47.80	0.98

HPTLC of Alcoholic extract of *C.spiralis*

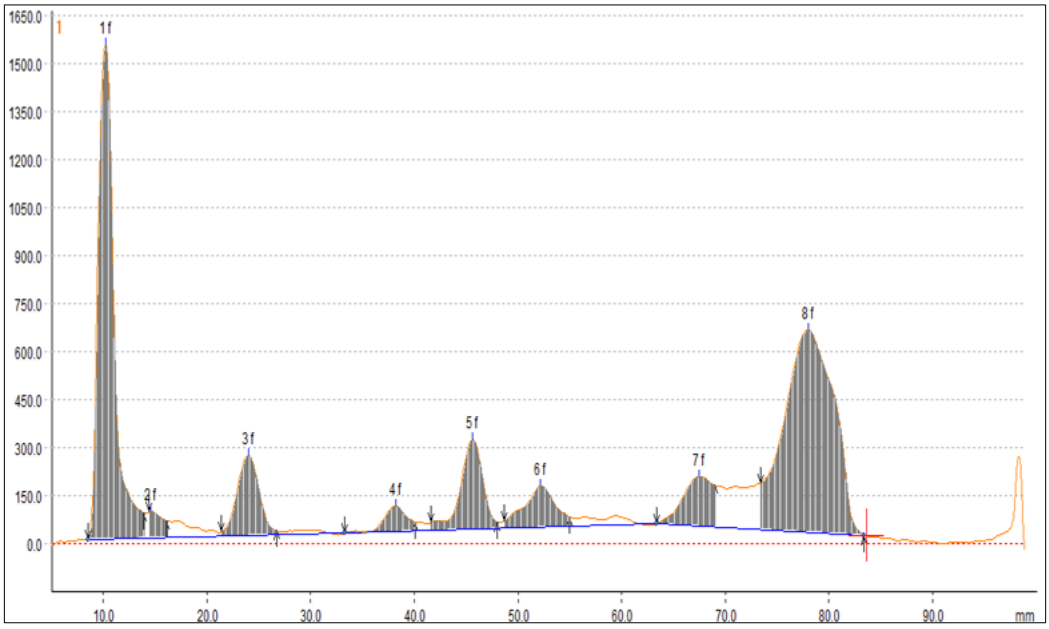


Fig 7b: Densitogram of Alcoholic extract of *C. spiralis* at UV 254nm

Table 8: Peak list of Alcoholic extract of *C.spiralis* at UV 254nm

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	10.2	2754.94	30.98	1543.43	0.02
2	14.6	118.83	1.34	84.03	0.08
3	24.0	617.89	6.95	250.51	0.21
4	38.2	205.96	2.32	81.73	0.40
5	45.6	709.71	7.98	280.00	0.49
6	52.2	458.64	5.16	130.53	0.58
7	67.5	510.11	5.74	155.89	0.83
8	78.0	3517.16	39.55	632.74	0.97

HPTLC of Alcoholic extract of *C. spiralis*

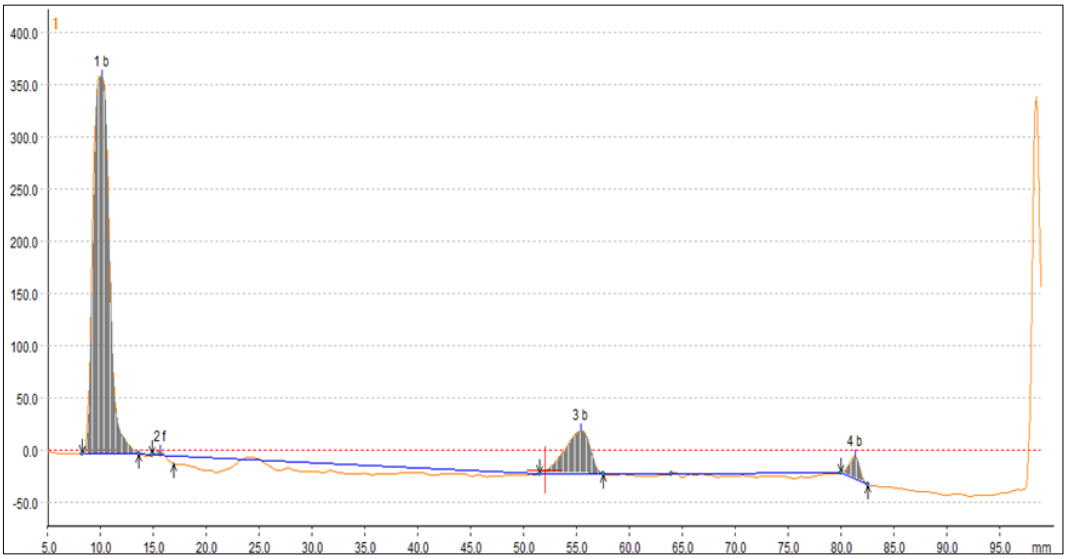


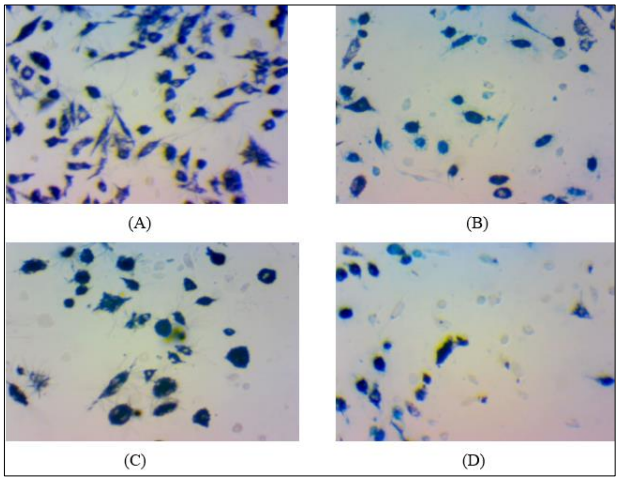
Fig 7c: Densitogram of Alcoholic extract of *C.spiralis* Upon exposure to Iodine vapour

Table 9: Peak list of Alcoholic extract of *C.spiralis* Upon exposure to Iodine vapour.

Peak no	Y-Pos	Area	Area %	Height	Rf value
1	10.1	653.03	82.00	361.92	0.02
2	15.6	3.27	0.41	4.57	0.09
3	55.4	115.88	14.55	41.53	0.64
4	81.3	24.24	3.04	21.73	1.00

Anticancer activity (Fig-8&9)

TheMDA-MB-231 (human breast cancer) cell lines were used for cytotoxicity analysis by reading formazan crystals formed by the reaction of mitochondrial dehydrogenase by MTT assay. At 48 h of time course incubation period, a significant abatement in cell viability was observed in the treated cell lines, while the concentration of tuber aqueous extract was increased from 10, 25, 50 and 100 µg/ml; and the DMSO was used as a positive control to exhibit 100% of healthy proliferated cells. At the 10 µg/ml concentration (IC_{50}) of tuber extract may have the capability to reduce 50% of treated cell lines when compared with negative control. From this study observed the tuberous extract exhibit strong cytotoxic activity against MDA-MB-231 cell lines.



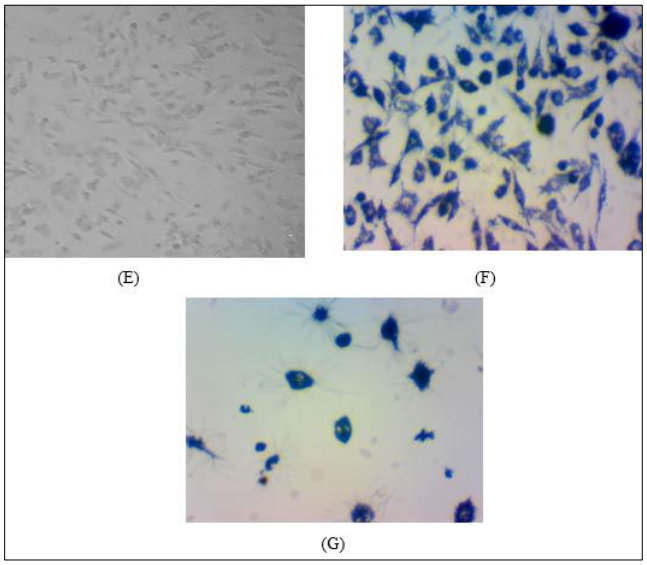


Fig 8: Anticancer activity of *C. spiralis* (A) 10 µg/ml, (B) 25 µg/ml, (C) 50 µg/ml, (D) 100 µg/ml, (E).untreated healthy cell lines, (F) DMSO (0.1%) negative control, (G) doxorubicin (1 lM) positive control.

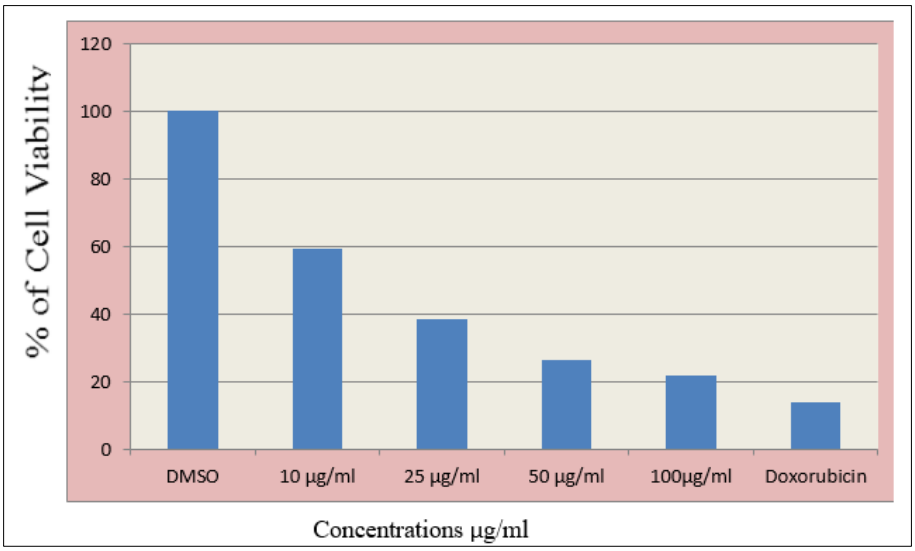


Fig 9: Anticancer activity of *C. spiralis*

Discussion

Most of the medicinal plants accumulate essential, important, necessary, useful and helpful elements for plant, man and animals. The presence of Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}, Co^{+3}, Cr^{+3}, Cu^{+3}, Fe^{+2}, Mn^{+2}, Ni^{+3} and Zn^{+2} reflects their function as essential nutrient elements, often as co-factor activators in metal-ligand enzyme complexes [39]. Ca +2 and Mg +2 are present in exchangeable amounts and act as binding agents to fuse the cell walls together [40]. The high concentration of certain metals, $Mg^{+2,}$ K^{+}, Ca^{+2} and Fe^{+2} in the plants are essential for proper growth and normal functioning of the plant [41]. Co^{+3}, Cr^{+3}, Cu^{+3} and Zn^{+2} are essential for hair growth and for increasing the rate of milk production for pregnant females [42-45].

Phenolic compounds are one of the most widely occurring groups which have considerable physiological and morphological importance in plants. These compounds participate in essential functions like reproduction and growth of plants and act as defense mechanisms against pathogens, parasites, and predators; also contribute to the color of plants [46]. These compounds exhibit a wide range of medicinal properties, such as anti allergenic, anti atherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardio protective and vasodilatory effects. Also acts as Antioxidant, anticancer, anticarcinogenic antimutagenic, antiatherosclerotic, l and antiviral activities [47-48].

Flavonoids are the largest group of water soluble phenolic compounds have great importance and application in pharmaceuticals and in food industry [49]. Flavonoids like malvidin, rosinidin, Delphidin, luteolinidin shows antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities. In plant systems, flavonoids help in combating oxidative stress and act as growth regulators [50].

Tannins are widely distributed in grape seed and skin and in pine bark, are considered to be the most potent antioxidants and frequently used in health care and cancer treatment. Traditional Chinese medicinal plants associated with anticancer tannin constituents (Gallotannins, ellagitannins, and proantho-cyanidins) with high levels of catechu [51]. A comparative study on *in vivo* and *in vitro* tuber extracts of *C. pusilla* confirmed anti proliferative property against HeLa

cancer cell line [52]. As well as the three *Ceropegia* species *C. spiralis*, *C. juncea* and *C. candelabrum*, screened for anti-cancer activity and confirmed the potent anticancer effect of ethyl acetate fraction of *C. spiralis* against HCT-118 Cell line (Colon cancer cell) [53].

Conclusion

Standardization of herbal drugs should be ensured to provide sound scientific footing to enhance consumer confidence and to improve business prospects for herbal medicines. The present work was thus planned to establish pharmacognostic standards of *C.spiralis* so as to have reliable parameters to authenticate the plant. Qualitative and Quantitative phytochemical analysis indicated the presence of steroids, triterpenoids, alkaloid, phenols, flavonoids, tannins, and saponins, Considerable amount of macro and micro elements are present in the plant. The presence of phytochemicals along with minerals can make *C.spiralis* a potential food and drug. The ash values of a drug give an idea about the presence of impurities like the earthy matter or the inorganic composition and other impurities. Extractive values are primarily useful for determination of exhausted or adulterated drug. The development of HPTLC fingerprints of alcoholic tuber extract of *C.spiralis* which can be used for identification, authentication and characterization. HPTLC technique is very crucial and important to detect the number of components in the extract and can provide quantitative aspects as well using the peak areas recorded in the densitogram obtained. It is the important parameter used for detecting adulteration to evaluate the quality of drugs. Major significance of HPTLC is its ability to analyze multiple samples simultaneously using small amount. The expository synergistic efficiency of *C.spiralis* aqueous extract activity on MDA MB231 (Human Breast) Cancer cell lines; Further studies needs to be performed to evaluate the molecular mechanism behind the anticancer potential of the *C.spiralis* aqueous extract against the Human Breast cancer cells. Thus, phytochemical analysis, ash value, extractive value, and anticancer analysis will be helpful in rapid identification of the drug.

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