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Phytochemical, Elemental, physico-chemical, and Anticancer investigations of Gynura lycopersicifolia DC.

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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 *Gynura lycopersicifolia* a medicinal plant was carried out. Qualitative and quantitative phytochemical analysis of whole plant 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 anti-inflammatory, antimicrobial and antioxidant activities leads to the isolation of new and novel compounds. Physicochemical reveals that dry matter 96.4% followed by Cellulose 25.34%. Elemental analysis reveals Potassium 8.92% followed by Calcium 2.82%. Along with this, macros, micro elements which are essential for maintaining the animal body were also determined quantitatively in whole plant found. Anticancer activity of *G. lycopersicifolia* exhibited potential anticancer activity towards HeLa (Human Cervix Adenocarcinoma) cancer cell lines, shows 74.39% cell death with cell viability 25.61% at 200 µg/ml. The presence of various bioactive compounds confirms the application of *Gynura lycopersicifolia* for various ailments by traditional practitioners.

Keywords: *Gynura lycopersicifolia* - Quantitative, Steroids, Alkaloids, Flavonoids, Phenols, HeLa (Human Cervix Adenocarcinoma).

I. INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and 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 number of 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 a raw drug [3]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [4]. Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer [5] and Alzheimer's 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 medicinal plant *Gynura lycopersicifolia* (Asteraceae) is a slender, succulent, erect herb; leaves deeply, irregularly pinnatifid and glabrous, 6-8×10-13cm, with white hispid pubescent, auricled at base. Flowers on head inflorescence homogamous, 1.5×0.5cm.light yellow disciform, receptacle flat; fruit Achenes, sparsely hispid between the ribs (**Fig. 1**) grown near streams in moist forests on high hills, distributed in India and in Srilanka. Flowering and fruiting December-February [11] it is commonly called as Adavi Tametaaku Chettu. The leaves used for anthelmintic, antiseptic, and for fever [12]. About 100 gm of leaf paste is made into pills of 5gm each is taken orally three times a day for patients suffering from post natal depression [13].

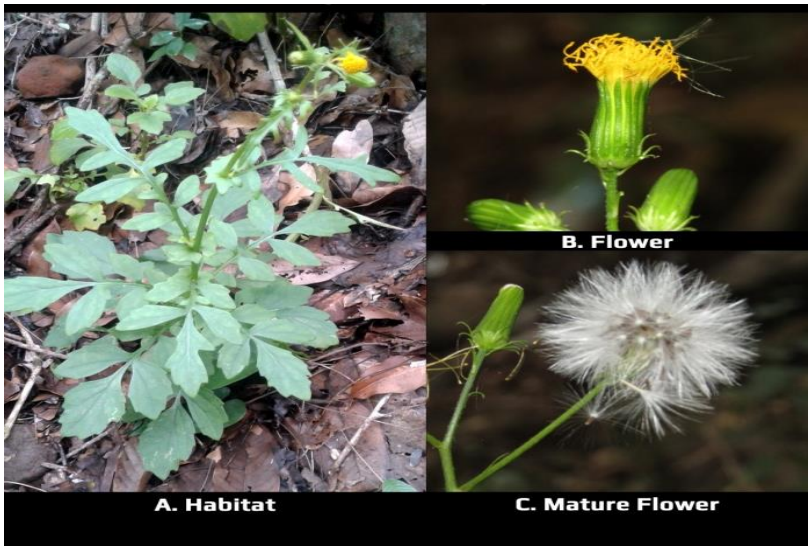


Fig 1: *Gynura lycopersicifolia*

II. MATERIAL AND METHODS

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of different solvents extracts of whole plant were carried out according to Standard methods [14-16]

Quantitative Phytochemical Analysis:

Determination of Total Tannin Content, total phenolic content and total flavonoid content was carried out by the following methods [17-21].

Elemental analysis:

Elemental analysis of the whole plant was done by the standard procedures [22].

PHYSICO CHEMICAL ANALYSIS:

Physico-Chemical analysis of the selected whole plant was done by the standard procedures [23-26]

Statistical analysis:

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

Anticancer activity:

Aqueous whole plant extract of *Gynura lycopersicifolia* was subjected to MTT 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye (MTT) to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectro photometrically at 570 nm [27-28]. HeLa cell line (Human Cervix Adenocarcinoma) is procured from National Centre for Cell Sciences (NCCS), Pune, India. The Dulbecco's Modified Eagle's Medium with

high glucose is used to growing up 2×10^4 cells per well in 96-well plates and 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* [29].

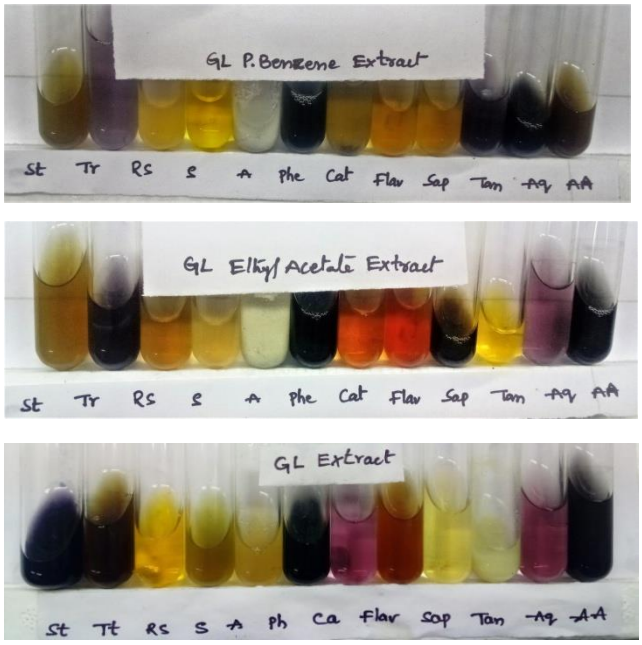
Afterwards medium was expelled and treated with different concentrations (12.5, 25, 50, 100 and 200µl/ml) Extracts of *Gynura lycopersicifolia* incubated for 24 hrs, further, remove the spent media and add 100 µl of MTT reagent with the 0.5mg/ml concentration and incubate the plate for 2.5 hrs for the reaction. Later, remove MTT reagent completely and add 100ul of 100% Dimethyl sulfoxide (DMSO) to solubilize the formazone crystals completely and measure the absorbance at 570 nm using 96 well Plate reader. The 0.1% of DMSO used to dissolve the nanoparticles and set as negative control and 15 µM *Camptothecin* treated cell lines were set as positive control. The initial experiment was maintained for 0 to 24 hrs of timeline period with 12 hrs 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 the results, significant cytotoxicity was observed at 24-hrs at 37^0C incubation period. The percentage of cell viability was calculated by the following formula [30]

$$\text{Percentage of Cell viability} = \frac{\text{OD value of treated cell lines}}{\text{OD value of control}} \text{ X } 100 \quad \rightarrow (1)$$

III. RESULTS

Preliminary phytochemical analysis

Results of the different phytochemical tests are presented in (Fig.2 & Table 1) Out of five extracts in Methanol (11) Aqueous (09), Ethyl acetate (6), Chloroform (4), Petroleum ether and benzene (4) secondary metabolites were observed. The important secondary metabolites are Alkaloids, Phenols, Flavonoids, Saponins and Aminoacids. But steroids are present only in Chloroform and Aqueous extracts. Sugars and Reducing sugars are present only in Methanol; Triterpenoids are present in Petroleum Benzene and in Methanol extracts. Similar types of results were observed in *Chromolaena odorata* [31] *Helianthus annuus* [32] of Asteraceae members.



St-Steroids, Tt-Triterpenoids, Rs- Reducing sugars, S-Sugars, Ph-Phenols, Ca-Catechin, Flav-Flavonoids Sap-Saponins, Tan. Tanins Aq-Anthraquinones, AA -Aminoacids.

Fig 2: Qualitative analysis of *G. lycopersicifolia*

Table 1: Qualitative Analysis of *G. lycopersicifolia:*

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

(+ Present, - absent)

Highest numbers of phytoconstituents are present in Methanolic extract followed by aqueous extract.

Quantitative Phytochemical Analysis:

Standard curve was prepared for the determination of total phenolic content and flavonoids using different concentrations of Gallic acid and quercetin respectively. Tannin content was calculated as a difference between total phenolics and non-tannin phenolic content. The total phenolics, flavonoids, tannins in different extracts of *G. lycopersicifolia* have been analysed in (Table 2a, 2b, 2c & Fig 3a, 3b, 3c.) Observation shows that the total phenolic content is highest in the Methanolic extract 48.33 ± 2.516 mg; followed by aqueous 30 ± 2.645 mg and significantly lower in the petroleum benzene ether and extracts 10.66 ± 1.52 mg. Similarly concentration of flavonoids is significantly high in aqueous 122.5 ± 3.307 mg of GAE/g of extract as compared to Methanol and Ethyl acetate extracts. However, the concentration of tannin content is significantly lower in chloroform extract 48.33± 1.572 mg of GAE/g of extract as compared to Methanol 80.208± 2.366 mg of GAE/g of extract. Similar type results was observed in *Artemisia absinthium* [33]

Table 2a: GL Total Flavonoid content

Extraction	Total flavonoid content (QE mg/g)
Petroleum benzene	37.916 ± 3.145
Chloroform	42.083 ± 1.909
Ethyl acetate	84.166 ± 2.602
Methanol	120 ± 2.5
Aqueous	**122.5 ± 3.307**

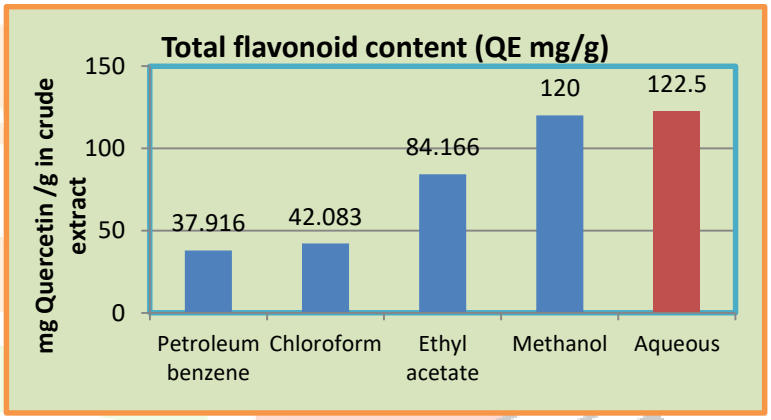


Fig 3a: Graphical representation of GL Total Flavonoid content

Table 2b: GL Total Phenolic Content

Sample	Total Phenolic content GAE mg/g
Petroleum benzene	10.66±1.52
Chloroform	26±1
Ethyl acetate	34±2
Methanol	**48.33±2.516**
Aqueous	30±2.645

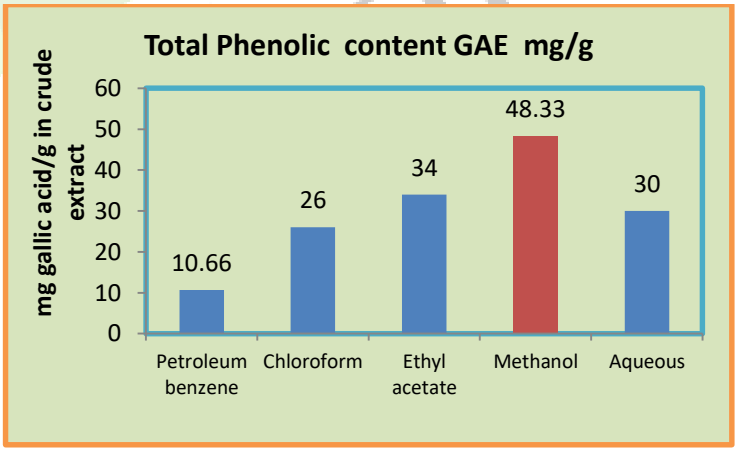


Fig 3b: Graphical representation of GL Total Phenolic Content

Table2c: GL Total Tannin Content

Sample	mg GAE/g
Petroleum benzene	61.25 ± 2.5
Chloroform	48.33 ± 1.572
Ethyl acetate	67.291 ± 1.909
Methanol	**80.208 ± 2.366**
Aqueous	52.916±2.009

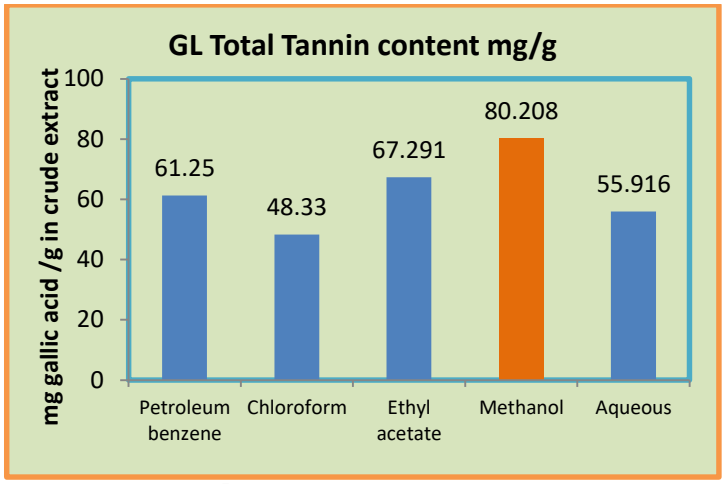


Fig 3c: GL Total Tannin Content

Elemental analysis

The results of elemental analysis (table 3) the macro elements highest percentage was found in Potassium 8.93; followed by Calcium 2.82; phosphorous having the lowest percentage 0.48; The microelements highest amount Iron 1281 ppm followed by Zinc 181 ppm lowest amount was record Molybdenum 27.80 ppm.

Table 3: Elemental (ICP - OES) analysis of ***G. lycopersicifolia***

Parameters	Unit	Reading
Lab reference		25
Your reference		GL
Nitrogen	%	3.1
Phosphorus	%	0.48
Potassium	%	8.93
Calcium	%	2.82
Magnesium	%	0.77
Zinc	ppm	181.8
Iron	ppm	1281
Copper	ppm	45.23
Manganese	ppm	127.4
Boron	ppm	124.3
Molybdenum	ppm	27.80

PHYSICO CHEMICAL ANALYSIS:

The results of Physico-Chemical analysis of the whole plant show (table 4, fig 4) dry matter having highest percentage 96.04; followed by cellulose 25.34; Acid insoluble ash having lowest amount 5.21; similar type of results was observed in *Curcuma neilgherrensis* [34] *Oryza sativa* [35]

Table 4: Physicochemical analysis of ***G. lycopersicifolia***

S.No	Parameters	Gl
1	Cellulose %	25.34
2	Hemicelluloses %	10.56
3	Lignin %	5.21
4	Dry matter %	96.04
5	Crude protein %	12.44
6	Loss on Drying at $105^{O}C$	6.09
7	Water soluble extractive	11.09
8	Alcohol soluble extractive	22.69
9	Acid insoluble ash	2.16
10	Total ash	18.31

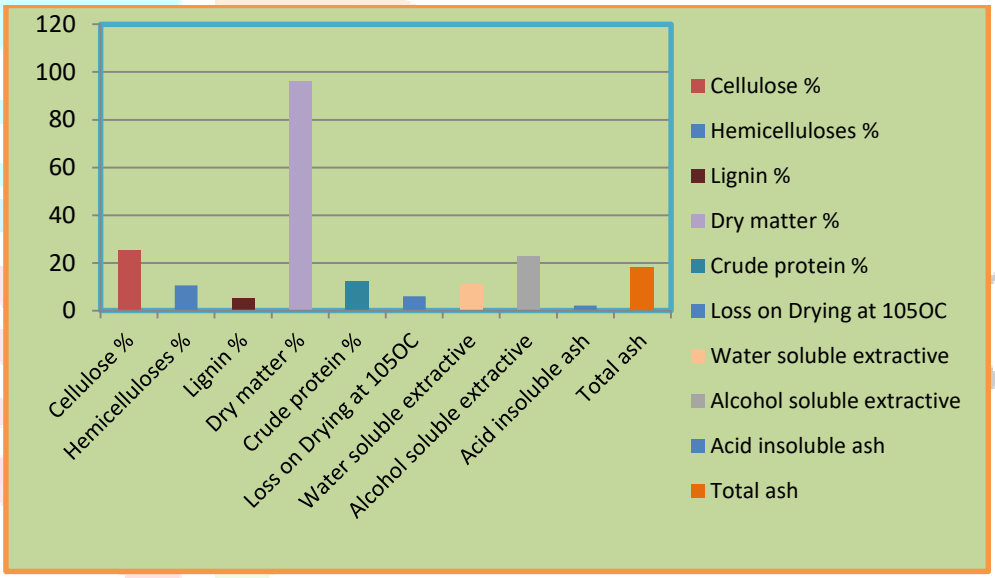


Fig 4: Graphical representation of physicochemical analysis of ***G. lycopersicifolia***

Anticancer activity

The HeLa cell lines (Human Cervix Adencarcinoma) were used for cytotoxicity analysis by formazan crystals formed by the reaction of mitochondrial dehydrogenase (MTT) assay. At 48 hrs of time course incubation period, a significant abatement in cell viability was observed in the treated cell lines. The concentration of *G.lycopersicifolia* aqueous extracts were used from 12.5, 25, 50, 100 and 200 µg/ml. Dimethyl sulfoxide (DMSO) was used as a positive control to exhibit 100% of healthy proliferated cells (**Figs. 5 & Table 5a & 5b**). The 50 µg/ml concentration (IC_{50}) of *G.lycopersicifolia* may have the capability to reduce 50% of treated cell lines when compared with negative control.

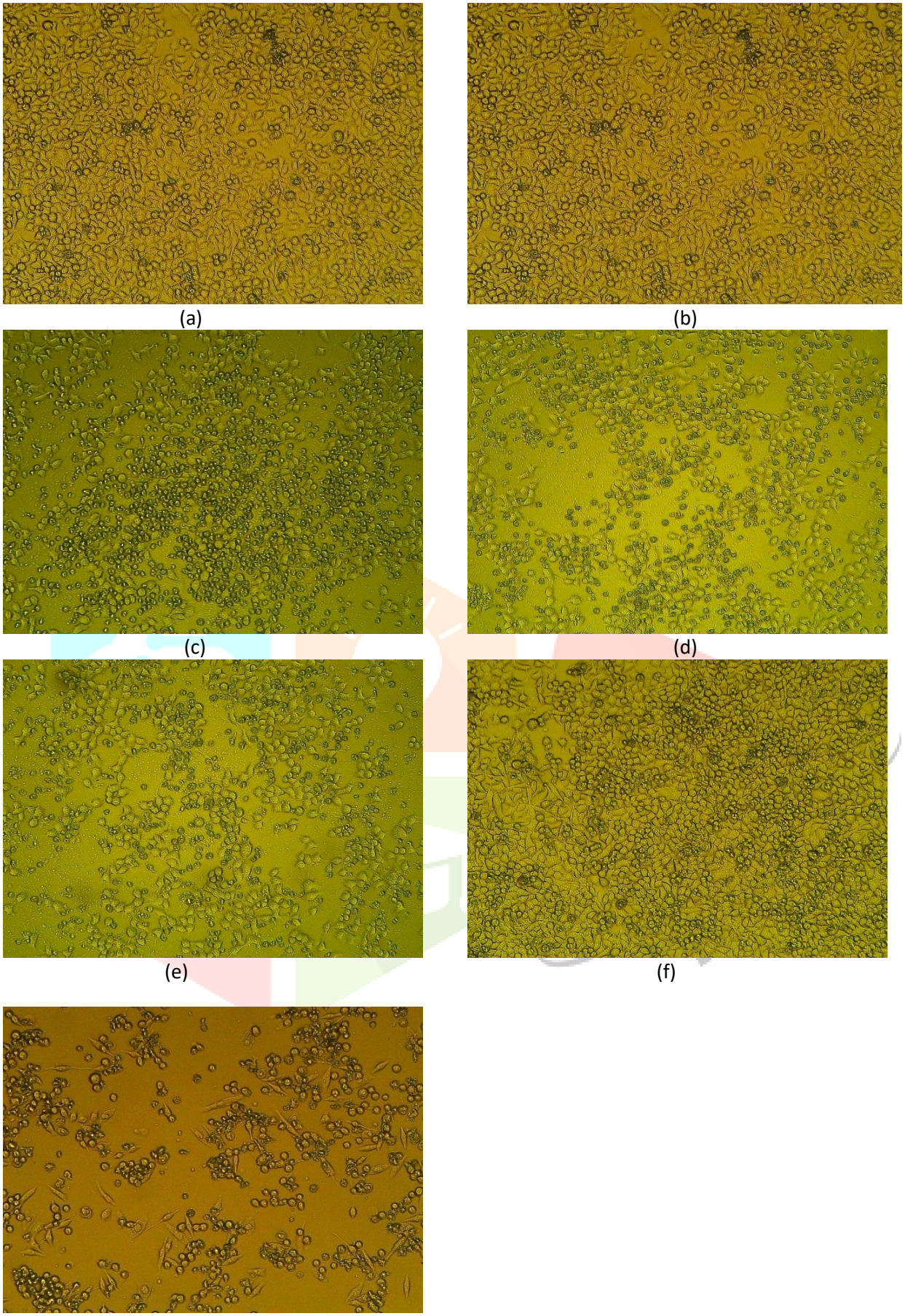


Fig 5a: Anticancer activity of *G. lycopersicifolia* (a) 12.5µl/ml, (b) 25 µl /ml,(c) 50 µl /ml, (d) 100 µl /ml, (e) 200 µl /ml, (f) DMSO (0.1%) negative control, (g) Camptothecin (5.2 µl /ml) positive control.

Table 5: Anticancer effect of ***G. lycopersicifolia*** extract on He La cell line (Human Cervix Aden carcinoma) by MTT assay.

S. No	Concentrations (µg/ml)	Absorbance (O.D)	Cell viability (%)	Cell Death (%)
1	DMSO	0.86	100	0
2	**12.5**	0.7385	87.42	12.58
3	**25**	0.5905	82.31	17.69
4	**50**	0.4335	76.23	44.07
5	**100**	0.316	45.47	54.53
6	**200**	0.16	18.25	81.75
7	**Camptothecin**	0.4425	13.19	86.81

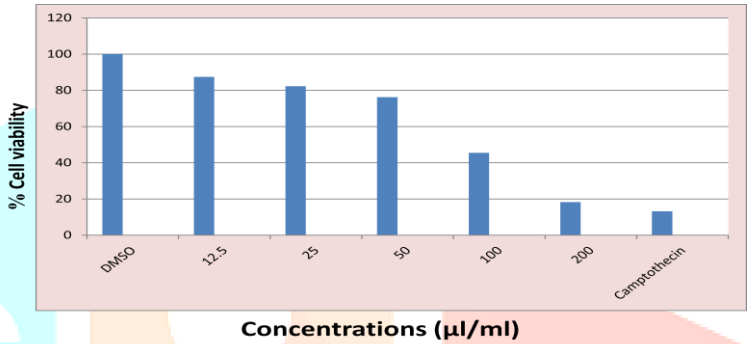


Fig 5b: Graphical representation of Anticancer activity.

IV. DISCUSSION

The WHO survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, for their primary healthcare [36]. These medicinal plants are rich sources for naturally occurring antioxidants especially phenolic and flavonoids contents. These agents have ability to scavenge free radicals, super oxide and hydroxyl radicals, etc thus they enhance immunity and antioxidant defense of the body [37].

Steroids and triterpenoids are pharmacologically active compounds and show the analgesic properties [38]. Alkaloids are beneficial chemicals to plants with predator and parasite repelling and physical state. Number of alkaloids was isolated from dicots and using as efficient drugs. Flavonoids are free radical scavengers and have strong anticancer activity [39], evidence of their inherent ability to modify the body's reaction to allergies, virus and carcinogens. Flavonoid rich species can play the role in pharmacological activities as anti-inflammatory, analgesic, antioxidant, antifungal and immune stimulant providing a key role of flavonoids to their biological actions [40].

The higher amounts of phenols are important in the regulation of plant growth, development and disease resistance. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolic compounds, especially their anti-inflammatory activity, antiviral and cytotoxic activity [41]. Traditionally saponins have been extensively used as detergents as pesticides and mollusicides, in addition to their industrial applications as foaming and surface active agents and also

have beneficial health effects [42]. Tannins contribute property of astringency that is fasten the healing of wound and inflamed mucous membrane and have receive considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [43].

Nitrogen is the element most absorbed in soil by plants growing under normal conditions. For this reason and due to their high mobility in soil, N is also the nutrient that is more deficient for most crops all over the world [44] Phosphate is the primary iron in extracellular and intracellular fluid; it aids absorption of dietary constituents, helps to maintain the blood at a slightly alkaline level, regulatory enzyme activity and is involved in the transmission of nerve impulses [45]**.** Potassium is the main intracellular cation and plays a role of primary importance in nerve and muscle excitability [46]. Calcium is the most abundant element in the animal body and it is fundamental for the activity of many enzyme systems, coagulation of blood, transmission of nerve impulses, contraction of muscles, flocculation of casein in the stomach and many other [47] Zinc deficiency is associated with mental impairments, lethargy, emotional disorder and irritability [48]. Iron is an essential mineral and an important component of proteins involves in oxygen transport and metabolism [49]. Copper (Cu) is universally important cofactor and activator of numerous enzymes which are involved in development and maintenance of the cardiovascular system. Manganese participates to the non-toxic function of the iron, vitamin C and the potentiating of the hypoglycemic effect of adrenaline [50].

A beneficial or even essential role of Boron in animal metabolism is supported by the findings that low B concentrations induce the MAPK pathway in cultured animal cells with a knockout of the B transporter Na B Cl, the mammalian homolog of At B or 1, stop to develop and proliferate [51]. Molybdenum is important essential trace element involved in metabolism through metalloenzymes [52].

Physico-chemical analysis of the whole and powdered drugs indicates identity, purity, and quality of herbal drugs [53].

V. 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 for *G. lycopersicifolia* 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 *G. lycopersicifolia* a potential food and drug. The ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present. Extractive values are primarily useful for determination of exhausted or adulterated drug. The expository synergistic efficiency of *G. lycopersicifolia* aqueous extract activity on HeLa (Human Cervix Adenocarcinoma) Cancer cell lines .Further studies need to be performed to evaluate the molecular mechanism behind the anticancer potential of the *G. lycopersicifolia* aqueous extract against the Human Cervix cancer cells. Thus, phytochemical

analysis, ash value, extractive value, and anticancer analysis will be helpful in rapid identification of the drug.

VI. ACKNOWLEDGEMENT

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