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PHYTOCHEMICAL SCREENING AND *INVITRO* ANTI CANCER ACTIVITYOF ETHANOL AND AQUEOUS *EXTRACTS OF COLDENIA PROCUMBENS LINN.*

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ABSTRACT

The main aim of present work evaluation of invitro anti-cancer activity of ethanol and aqueous extracts of Coldenia Procumbens linn. Extracts were subjected to various qualitative phytochemical tests to identify the active constituents which showed presence of Alkaloids, Glycosides, Saponins, Carbohydrates, Tannin & Phenolic compounds, Triterpenoids, Steroids, Flavonoids, Fixed oil and fats. Ethanolic extract showed the presence of Alkaloids, Glycosides, Saponins, Carbohydrates, Tannins & Phenolic compounds Triterpenoids, Steroids, Flavonoids, Fixed oil and fats, Gum and mucilage and Lignin's, whereas the aqueous extract showed, presence of Glycosides, carbohydrates, Tannin, Phenolic compounds, Flavonoids and Lignin's. The ethanolic and aqueous extracts of *Coldenia procumbens* found to possess moderate cytotoxic potential with reference to the standard drug Methotrexate against MCF-7 breast cancer cell line. Among the extracts, the ethanolic extract showed better activity than the aqueous extract when comparing with standard. The reported cytotoxic activity of the plant extracts in the present study may be due to thepresence of phenolic and flavonoid constituents. This indicates the possibility of the plant extracts investigated for further development to cancer therapeutic agent and warrants further studies to understand the mechanisms of cytotoxic activity of the plant extract *Coldenia procumbens*.

KEYWORDS: Evaluation, Invitro, Anti-Cancer Activity, Ethanol And Aqueous Extracts, Coldenia Procumbens Linn.

INTRODUCTION

The burden of cancer rose to 18.1 million new cases and 9.6 million deaths in 2018. With 36 different types, cancer mainly affects men in the form of colorectal, liver, lung, prostate, and stomach cancer and women in the form of breast, cervix, colorectal, lung, and thyroid cancer.[1] Treating cancer has become a whole new area of research. There are conventional as well as very modern techniques applied against cancers. A variety of techniques i.e., chemotherapy, radiation therapy, or surgery are used for treating cancer. However, all of them have some disadvantages.[2] The use of conventional chemicals bears side effects and toxicities.[3] But as the problem persists, new approaches are needed for the control of diseases, especially, because of the failure of conventional chemotherapeutic approaches. Therefore, there is a need for new strategies for the prevention and cure of cancer to control the death rate because of this disease.

Herbal medicine has become a very safe, non-toxic, and easily available source of cancer-treating compounds. Herbs are believed to neutralize the effects of diseases in a body because of various characteristics they possess.[4] For instance, among the many anticancer medicinal plants, *Phaleria macrocarpa* (local name: *Mahkota dewa*) and *Fagonia indica* (local name: *Dhamasa*) have been used traditionally for the anticancer properties of their active ingredients.[5,6] Metabolites extracted from the plant material are used to induce apoptosis in cancer cells. Gallic acid as the active component was purified from the fruit extract of *P. macrocarpa* and has demonstrated a role in the induction of apoptosis in lung cancer, leukemia, and colon adenocarcinoma cell lines.[7,8] It is a polyhydroxy phenolic compound and a natural antioxidant that can be obtained from a variety of natural products i.e., grapes, strawberries, bananas, green tea, and vegetables.[9] It also plays a critical role in preventing malignancy transformation and the development of cancer.[10] Similarly, other compounds such as vinca alkaloids, podophyllotoxin, and camptothecin obtained from various plants are used for the treatment of cancer**.**

With the advancement in the industrial sector and industrial medicine, the use of herbs was forgotten for a

long period of time.[11] Hurdles regarding natural compounds are reduced because of the advent of new techniques and interest has been developed in the use of such natural ingredients in the pharmaceutical industry.[12,13] It has been estimated by the world health organization that 80% of the world is using traditional treatment methods.[14] Understanding of the effects or actions of herbs on various targets comes with the help of modern biomolecular science which recognizes some important properties i.e., anticancer, anti-inflammatory, and anti-virus. With the increasing understanding of the effects of such herbal medicine, their effects against different types of cancers have also been identified. For instance, hepatocellular carcinomas (HCC) are considered as the fifth most common malignancy in the world with increasing incidence.[15,16] Many studies have been performed on the treatment and prevention of using herbal medicine against HCC in which it is shown that all phases of HCC such as initiation, promotion, and progression could be affected by components of herbs.[17,18]

However, as far as herbal compounds are considered as drugs, it is erroneously believed that they have no issues in terms of safety and side effects. There are hundreds of species of plants that are toxic to health. In the same way, there are many compounds in otherwise friendly plants that cause cytotoxicity. Based upon testing it has been proved that even anticancer plants result in cytotoxic effects.[19]

Coldenia procumbens is a Procumbent deep rooted, hairy herb wide spread in tropical and sub tropical Africa, Asia and Australia, found throughout India as a weed in moist place. It is often found in seasonally flooded locations, e.g. on dry rice fields, where it is a common weed, but it can also withstand severe drought. It used as external application for causing suppuration of boils. In folklore medicine it is used to treat rheumatic swellings, leucorrhoea, menorrhagia, anti-diabetic, anti-arthritic and hypotensive. Pain is associated with a wide range of injury and disease, and is sometimes the disease itself. Some conditions may have pain and associated symptoms arising from a discrete cause, such as post operative pain or pain associated with a malignancy, or may be conditions in which pain constitutes the primary problem, such as neuropathic pains or headaches. In view of traditional uses of Coldenia and the Phytochemical review[20] & Pharmacological activities like anti-inflammatory[21,22] hepatoprotective[23], antibacterial[24] & anthelminthic[25] activities reported on this plant the present study has been undertaken to investigate the analgesic activity of methanolic extract of Coldenia procumbens.

The main aim of present work evaluation of invitro anti-cancer activity of ethanol and aqueous extracts of Coldenia Procumbens linn.

MATERIALS AND METHODS

Fresh mixture were **collected** and was dried. After drying they were again pulverized. The size is reduced. The dried plant *Coldenia procumbens*, powder mixture was weighed about 250g Extracted by soxhlet apparatus using 99% of ethanol and water as a solventfor 72 hours. The yield of product was 7.358g.

Collection of Specimen

The species for the proposed study that is *Coldenia procumbens*, Gagnep.family Boraginaceae was collected from surroundings of Thirupathi.

PRELIMINARY PHYTOCHEMICAL TESTS

The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as Carbohydrates, Protein and Lipids that are utilized as food by men, but also for a multitude of compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc., that exerts a physiologic effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents.

For our present study, we had taken the plant material as powdered plant of *Coldenia procumbens.* To extract the compounds are tested the chemical constituents present in them.

PREPARATION OF EXTRACTS

Ethanol extract

The shade dried course powder of the entire plant (250 gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with 99.99% ethanol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. Dried and kept in a desiccator till experimentation. Obtained extract (dark blackish brown) was weighedand percentage yield was calculated in terms of air-dried powdered crude material (ethanolic extract was named as ETE).

Aqueous extract

The shade dried course powder of the entire plant (250 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with distilled water until the completion of extraction. The extract was filtered while hot and the resultant extract was distilledin vacuum under reduced pressure in order to remove the distilled water completely. It was finally dried and kept in a desiccator till experimentation. Obtained extract (dark reddish brown) was weighed and percentage yield was calculated in terms of air-dried powdered crude material (ethanolic extract was named as AQE).

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Both ethanolic and aqueous extracts obtained from the powdered plant *Coldenia procumbens*. were subjected to various qualitative tests for the identification of various plant constituents present in this species.

INVITRO ANTICANCER STUDIES

Among the various diseases attributed to mortality in humans all over the world, cancer is a leading cause. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and Plants have a long history of use in the treatment of cancer. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. Over the past few decades, a significant progress has been made in cancer prevention and treatment. Plant-derived natural products are becoming important as anti-cancer derivatives, including vincristine, vinblastine, paclitaxel and camptothecin, which are invaluable contributors of nature to modern medicine.

In recent years, *in vitro* toxicology has rapidly developed into a challenging new scientific discipline. Some of the complications occur during *in-vivo* cytotoxic screening that is intravenous administration of chemotherapeutic drugs cause significant individual differences in biotransformation and bio-distribution. To overcome this problem, *in-vitro* cytotoxic screenings are used in which the effect of chemotherapeutic drug is being studied in the tumor cells in culture outside the body. Also, *in vitro* toxicity tests have gained the support of many animal welfare organizations, and are seen as one of the most promising means whereby the reduction and replacement of animal usage can be achieved.

Principle

The principle of this colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically (174-175). Only viable cells with active mitochondria reduce significant amounts of MTT since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Media

Leibovitz L-15 Medium with L-Glutamine, FBS (Fetal Bovine Serum, SFM HEK-293 (Serum Free Media), Thioglycollate medium (TGM), Tryptone soya broth (TSB) and Cellproliferation kit (MTT) 1000 tests.
Cell lines: MCF-7 (Breast cancer cell line) was purchased from NCCS, Pune.

Cell treatment Procedure

Cytotoxicity of the plant extract on the MCF-7 breast cancer cell lines was determined using the MTT Proliferation assay kit. The cells in a concentration of 1×10 cells/ml were preincubated in culture medium for 3 hrs at 37 °C and 6.5 % CO_2. The cells were seeded at a concentration of 5×104 cells/well in 100 µl culture medium and at various concentrations (5 -100 µg/ml) of standard Methotrexate and extract (dissolved in 2 % DMSO (dimethyl sulphoxide) solution) into microplates (tissue culture grade, 96 wells, flat bottom) and incubated for 24 hrs at 37 °C and 6.5 % CO_2.

The test denotes the survival cells after toxic exposure. Then, 10 µl MTT labeling mixtures were added and incubated for 4 hrs at 37 °C and 6.5 % CO_2. Each experiment was done in triplicates. Then 100µl of solubilisation solution was added into each well and incubated for overnight. The spectrophotometric absorbance of the samples was measured using a microplate (ELISA) reader at a wavelength in between 550 and 600 nm according to the filters available for the ELISA reader. The reference wavelength should be more than 650 nm. Percentage inhibition of extract against all cell lines was calculated using the following formula.
% of cell survival = AT/AC x 100 AT– Absorbance of test
AC – absorbance of control
% of cell inhibition = 100 - % cell survival

The IC_{50} value, i.e., the concentration required to inhibit 50% of cell viability was determined by plotting the log of the drug concentration versus the percentage of inhibition. The best-fit line was plotted by least-squares linear regression. The 50% inhibitory concentration (IC_{50}) was calculated from the linear-regression equation: Log (CV_{50}) = m×log (IC_{50})+c; where m is the regression coefficient, c is the intercept of the line, log (IC_{50}) is the log of the 50% inhibitory concentration of the extract and log (CV_{50}) is the log value of 50% cell viability.

RESULTS AND DISSCUSSION

The plant *Coldenia procumbens* belonging to family Boraginaceae was selected for theproject. On the basis of ethanobotanical information, which reveals its uses against disease like wound, inflammation, fever, tumor etc. Literature survey showed that very less work has been performed on this plant. So we can validate scientifically for folk claim for its therapeutic activity. We have also undertaken its detailed, preliminary phytochemical and *invitro* pharmacological investigation to give an appropriate identification and rationalize its use as drug of therapeutic importance.

Preliminary phytochemical studies performed by starting with purification of solvents. Then powdered whole plant *Coldenia procumbenc*e subjected for continuous hot extraction with ethanol and distilled water. The yield was found to be 10.24 %w/w for ethanolic extract (ETE) and 16.2%w/w for aqueous extract (AQE). These extracts were subjected to various qualitative phytochemical tests to identify the active constituents which showed presence of Alkaloids,

Glycosides, Saponins, Carbohydrates, Tannin & Phenolic compounds, Triterpenoids, Steroids, Flavonoids, Fixed oil and fats. Ethanolic extract showed the presence of Alkaloids, Glycosides, Saponins, Carbohydrates, Tannins & Phenolic compounds Triterpenoids, Steroids, Flavonoids, Fixed oil and fats, Gum and mucilage and Lignin's, whereas the aqueous extract showed, presence of Glycosides, carbohydrates, Tannin, Phenolic compounds, Flavonoids and Lignin's.

The yield and % yield of both ethanolic and aqueous extracts of powdered plant of *Coldenia procumbens* were reported.

Table No. 1: Extraction Values Of Ethnolic And Aqueous Extracts.

S. No.	Extracts	Yield (gms.)	% Yield (w/w)
1.	Ethanol Extract (ETE)	25.60	10.24
2.	Aqueous Extract (AQE)	40.64	16.2

Table No. 2: Data For Preliminary Phytochemical Analysis Of Ethanolic And Aqueous Extracts Ofextracts Of *Cayratia Trifolia*gagnep.

Phytoconstituents	Ethanolic extract	Aqueous extract
Alkaloids	+	-
Saponins	+	-
Glycosides	+	+
Carbohydrates	+	+
Tannins,Phenolic compounds	+	+
Flavonoids	+	+
Steroids	+	+
Proteins and Amino acids	-	-
Triterpenoids	+	-
Fixed Oils and Fats	+	-
Gums and Mucilage	+	-
Lignins	+	+

***In vitro* cytotoxic studies against MCF-7 breast cancer cell line by MTT assay**

Cytotoxicity potential of AQE and ETE, extracts were determined using MTT assay against MCF-7 breast cancer cell line. A significant increase in the % of cytotoxic value of the AQE and ETEtreated cells were noted when compared to the standard. The IC50 for cytotoxicity was found to be the standard was 29.68µg/ml and cells treated with ETE were 37.51 µg/ml being the most potent inhibitor. AQE treated cells indicated an IC50 value of 40.6 µg/ml. The highest percentage inhibition was found to 97%, 92% and 86% for Standard drug Methotrexate, Aqueous Extract and Ethanolic Extract respectively. However, the percentage inhibition ofcytotoxicity was found to be lower for both the extracts AQE and ETE when compared to the standard drug methotrexate. In addition, ETE showed better inhibition than the AQE. The R^2value for the standard, AQE & ETE were 0.9755, 0.9560 & 0.9485 respectively.

Table No. 3: Absorption Data Of Std, Aqe & Etetable.

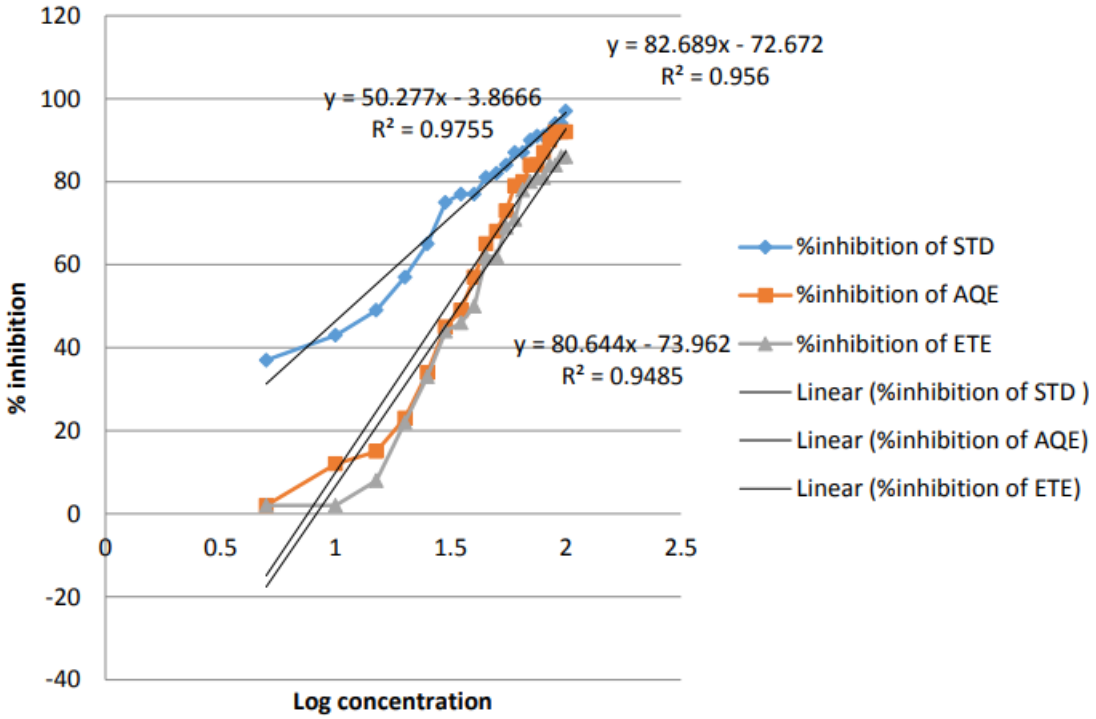
Absorbance at 570 nm STD	Absorbance at 570 nm AQE	Absorbance at 570 nm ETE
0.2469	0.0041	00042
0.1646	0.0457	0.0042
0.1881	0.0582	0.0295
0.2195	0.0915	0.0885
0.2508	0.1373	0.1349
0.2900	0.1831	0.1812
0.2979	0.1997	0.1897
0.2979	0.2330	0.2065
0.3136	0.2663	0.2571
0.3175	0.2788	0.2571
0.3253	0.2996	0.2866
0.3371	0.3246	0.2951
0.3371	0.3287	0.3246
0.3488	0.3454	0.3330
0.3528	0.3454	0.3372
0.3528	0.3579	0.3372

0.3567	0.3704	0.3499
0..3645	0.3787	0.3499
0.3645	0.3787	0.3583
0.3920	0.4162	0.4216

Table 4: Cytotoxic Activity Of Standard And Extracts Against Mcf-7Breast Cancer Cell.

Con. (µg/ml)	Log.Con	%Inhibition of STD	%Inhibition of AQE	%Inhibition of ETE
5	0.69897	37	2	2
10	1	43	12	2
15	1.17609	49	15	8
20	1.30103	57	23	22
25	1.39794	65	34	33
30	1.47712	75	45	44
35	1.54407	77	49	46
40	1.60206	77	57	50
45	1.65321	81	65	62
50	1.69897	82	68	62
55	1.74036	84	73	69
60	1.77815	87	79	71
65	1.81291	87	80	78
70	1.8451	90	84	80
75	1.87506	91	84	81
80	1.90309	91	87	81
85	1.92941	92	90	84
90	1.95424	94	92	84
95	1.97972	94	92	86
100	2	97	92	86

Cytotoxic activity of standard and extracts against MCF-7 breast cancer cell line.



***Invitro* cytotoxic studies on STD, AQE & EET (Fig. 18)**

Table 5: Linear Equation, R^2 and Ic50 Values Of Std, Aqe & Ete.

S.No.	Sample	Linear equation	R^2	IC_{50} value
1	STD (Methotrexate)	Y = 50.277*x- 3.866	0.9755	29.68µg/ml
2	AQE	Y = 82.689*x-72.67	0.9560	40.6 µg/ml
3	ETE	Y = 80.644*x-73.96	0.9485	37.51µg/ml

The compounds of Tannins & Phenolic compounds Triterpinoids, Steroids and Flavonoids have been reported in ETE. The literatures proved that these compounds are potent antioxidants and free radical scavengers. The antioxidant, antimicrobial, and antitumor activities due to its phenolic, flavanoid and aromatic compounds. These beneficial substances can act as antioxidants and electrophile scavengers, stimulate the immune system, form the DNA addicts with carcinogens and induce detoxification enzymes. Hence, the reported cytotoxic activity of ETE may be due to the presence of polyphenolic compounds and antioxidant potential of the extracts.

CONCLUSION

The ethanolic and aqueous extracts of *Coldenia procumbens* found to possess moderate cytotoxic potential with reference to the standard drug Methotrexate against MCF-7 breast cancer cell line. Among the extracts, the ethanolic extract showed better activity than the aqueous extract when comparing with standard. The reported cytotoxic activity of the plant extracts in the present study may be due to the presence of phenolic and flavonoid constituents. This indicates the possibility of the plant extracts investigated for further development to cancer therapeutic agent and warrants further studies to understand the mechanisms of cytotoxic activity of the plant extract *Coldenia procumbens*. However, the isolation of active principle will be advantageous to produce novel bioactive constituent from this extract which may possess more significant activity.

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