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STUDIES ON INVITRO ANTICANCER ACTIVITIES OF ACTIVE PRINCIPLES FROM CAYRATIA PEDATA

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ABSTRACT: Cayratia pedata is an indigenous medicinal plant commonly known as Birdfoot Grapevine. The leaves of this plant have been used as a traditional medicine for the treatment of ulcers, diarrhea and inflammation. The crude extracts of the plant has anti-microbial, anti-ulcer, anti-inflammatory and anti-diarrheal properties. The present study reports on the bioactive principles obtained from Cayratia pedata through extraction, isolation and identification. The photochemical screened and quantitative analyses were done by biochemical tests. The preliminary analyses reveal the presence of tannins, flavonoids, terpenoids, alkaloids, carbohydrate, saponins, steroids, quinines, phenols, proteins, oils and fats, phytosterols, coumarins and phlobatannins. The ethanolic and aqueous extracts of Cayratiapedata Gagnep were 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. .

Keywords: Cayratia pedata, photochemical, bioactive principles, Methotrexate.

Cancer is the abnormal growth of cells in our bodies that can lead to death. Cancer cells usually invade and destroy normal cells. These cells are born due to imbalance in the body and by correcting this imbalance, the cancer may be treated. Billions of dollars have been spent on cancer research and yet we do not understand exactly what cancer is. Every year, millions of people are diagnosed with cancer, leading to death. According to the American Cancer Society, deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide. Thus cancer kills about 3500 million people annually all over the world. Several chemo preventive agents are used to treat cancer, but they cause toxicity that prevents their usage10.In India, there are at least 250,000 species of plants out of which more than one thousand plants have been found to possess significant anticancer properties. Cancer is often deadly and affects a considerable number of people worldwide. Ongoing research is being done throughout the world to seek out effective treatments for cancer, including the use of plants to relieve and treat cancer patients. This treatment makes use of the compounds naturally present in plants that are known to inhibit or kill carcinogenic cells.

An alternative to chemotherapy, which is the most common means by which doctors and specialists treat cancer, organically based treatments may not have the severe side effects that radial treatments and chemotherapy has. The harsh side effects of cancer treatments are one motivating factor to finding alternative methods. The use of botanical when treating cancer patients is considered a natural alternative, because some plants may contain properties that naturally have the ability to prevent the spread or risk of developing various forms of cancer. As in all medical testing, careful precautions and considerations are taken when studying the different compounds present in plants that are known to treat cancer. Cayratia pedata (Lam.) Gagnep. Var. glabra Gamble (Family: Vitaceae) (Fig. 1) is a weak climber commonly known as kattuppirandai, ainthilai kodi (5-pedata) in tamil, goalilata in hindi, godhapadi in sanskrit and velutta sori valli in Malayalam. This species can be found in Thiashola and Korakundah range and scrambling over the hedges and trees. Vitaceae consist of approximately 14 genera and about 900 species primarily distributed in tropical regions in Asia, Africa, Australia, the neotropics and the Pacific islands.

The present work focused the comprehensive study of cayratia Pedata (wall.ex.wight) gengap. The ethno botanical review showed that the entire plant has potent medicinal properties such as wound healing, analgesic, anti-inflammatory, antiulcer and antitumor properties. Till now, few photochemical studies have been done on this plant. Therefore, this plant having wide scope for detail pharmacogonostical, preliminary photochemical and pharmacological investigation.

Figure 1. CayratiaPedata

MATERIALS AND METHODS

PROCEDURE:

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

IDENTIFICATION OF PHYTOCHEMICAL CONSTITUTENTS 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 photochemical screening for the detection of various plant constituents.

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

PREPARATION OF EXTRACTS

Preparation of the extracts of *Cayratiapedata* Gagneppowdered plant by using following solvents:

(a) Ethanol

(b) Distilled Water

(a) Ethanol extract

The shade dried course powder of the entire plant (250 gm) was packed well in sox let 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. Obtained extract (dark blackish brown) was weighed and percentage yield was calculated in terms of air-dried powdered crude material (ethanolic extract was named as ETE).

(b) Aqueous extract

The shade dried course powder of the entire plant (250 gm) was packed well in sox let 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 distilled in vacuum under reduced pressure in order to remove the distilled water completely. 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 *Cayratiapedata* gagnep .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 million persons living with cancer around the world (166). There is a necessity for search of new compounds with cytotoxic activity as the treatment of cancer with the available

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anticancer drugs is often unsatisfactory due to the problem cytotoxicity to the normal cells. Plants have a long history of use in the treatment of cancer. Several studies have been conducted on herbs under a multitude of ethno botanical 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[38-41].

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.

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), Try tone soya broth (TSB) and Cell proliferation kit (MTT) 1000 tests.

Cell lines:

MCF-7 (Breast cancer cell line) was purchased from NCCS, Pune. Cell treatment procedure45-48:

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 1Ã—10 cells/ml were preincubated in culture medium for 3 hrs at 37 Â°C and 6.5 % CO2. The cells were seeded at a concentration of 5Ã—104 cells/well in 100 Î¼l culture medium and at various concentrations (5-100 µg/ml) of standard Methotrexate and extract (dissolved in 2 % DMSO (dimethyl sulphoxide) solution) into micro plates (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 micro plate (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 Cayratiapedata Gagnep belonging to family Vitaeceae was selected for the project. 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 undertakes its detailed, preliminary photochemical and invitro pharmacological investigation to give an appropriate identification and rationalize its use as drug of therapeutic importance.

Preliminary photochemical studies performed by starting with purification of solvents. Then powdered whole plant

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Cayratiapedata Gagnepwere subjected for continuous hot extraction with ethanol and distilled water. The yield was found

to be 16.2 %w/w for ethanolic extract (ETE) and 10.24%w/w for aqueous extract (AQE). These extracts were subjected to various qualitative photochemical tests to identify the active constituents which showed presence of Alkaloids, Glycosides, Saponins, Carbohydrates, Tannin & Phenolic compounds, Triterpinoids, Steroids, Flavonoids, Fixed oil and fats. Ethanolic extract showed the presence of Alkaloids, Glycosides, Spooning, Carbohydrates, Tannins & Phenolic compounds Triterpinoids, Steroids, Flavonoids, Fixed oil and fats, Gum and mucilage and Lignins ,whereas the aqueous extract showed, presence of Glycosides, carbohydrates, Tannin, Phenolic compounds, Flavonoids and Lignins. The yield and % yield of both ethanolic and aqueous extracts of powdered plant of CCayratiapedata gagnep were reported.

TABLE NO. 1 - Extraction Values Of Ethnolic And Aqueous Extracts Ofcayratia Pedata Gagnep.

S. No.	Extracts	Yield (gms.)	% Yield (w/w)
1.	Ethanol Extract (ETE)	40.64	10.24
2.	Aqueous Extract (AQE)	25.6	16.2

TABLE NO. -2 Data For Preliminary Phytochemical Analysis Of Ethanolic And Aqueous Extracts Ofextracts Of *Cayratia Pedata 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 andETEtreated 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 of cytotoxicity 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, 0.9460 & 0.9585 respectively.

TABLE: 3 ABSORPTION DATA OF STD, AQE & ETE

Absorbance at 570 nm STD	Absorbance at 570 nm ETE	Absorbance at 570 nm AQE
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	0.4262	0.4116

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TABLE. 4

CYTOTOXIC ACTIVITY OF STANDARD AND EXTRACTS AGAINST MCF-7 BREAST CANCER CELL

Con. (µg/ml)	Log Con	%inhibition of STD	%inhibition of ETE	%inhibition of AQE
5	0.69897	37	2	2
10	1	43	22	2
15	1.17609	49	35	8
20	1.30103	57	49	22
25	1.39794	65	55	33
30	1.47712	75	66	44
35	1.54407	77	67	46
40	1.60206	77	69	50
45	1.65321	81	75	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.**

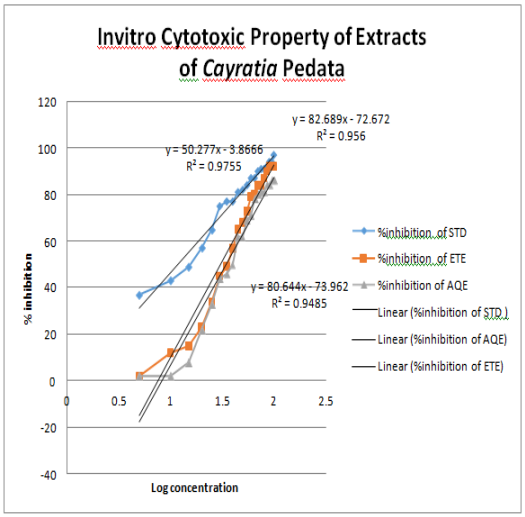

TABLE.5

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 andaqueous extracts of Cayratiapedata Gagnep belonging to family Vitaeceae were 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 falconoid 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 Cayratiape data Gagnep,. 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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