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PHYTOCHEMICAL INVESTIGATION AND CYTOTOXIC SCREENING OF THREE MEDICINAL PLANTS WHICH ARE TRADITIONALLY USED IN TREATING CANCER IN AYURVEDIC MEDICINE

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ABSTRACT

The current research was undertaken for phytochemical investigation and cytotoxic screening the root powder of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dillenii* which are traditionally used in treating cancer in Ayurvedic system of medicine. The phytochemical screening of petroleum ether, chloroform and methanol extracts of roots exhibited that all the three roots extracts contain carbohydrates, gums, fats and oil, sterols, alkaloids, flavonoids, tannins and phenolic compounds as major chemical constituents. Methanol and chloroform extracts contain the major phytoconstituents like alkaloids, flavonoids, and phenolic compounds. The cytotoxic potential of *Ficus heterophylla, Artocarpus lakoocha* and *Opuntia dilleni* roots extracts were evaluated by using *Allium cepa* root meristem assay. Various concentrations of test solutions were prepared by serial dilution method. Distilled water was used as the control. The methanol extracts revealed significant root growth inhibition in *Allium cepa* after 72h and also showed superior decrease in the mitotic index percentage whereas the petroleum ether extract manifested the mild cytotoxic activity. The cytotoxic activity raised with increase in concentration and the time of exposure to the test solutions. Consequently, the methanol extracts of the root powder of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dillenii* displayed remarkable cytotoxic potential which can arouse the anticancer activity.

Keywords: *Ficus heterophylla, Opuntia dillenii, Artocarpus lakoocha,* cytotoxic, *Allium cepa,* mitotic index

Key Words: ***Pseudomonas aeruginosa,*** **IAA production, PBD, Process optimization**.

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INTRODUCTION

Herbal medicine has its origins in ancient cultures including those of India, Egypt and Chinese. The medicinal plants are involved in the treating of disease and enhancing health care. In fact, many pharmaceutical drugs are also based on the synthesised natural compounds obtained from plants such as the heart drug digitalis was derived from the herb Foxglove or the anticancer drug vincristine extracted from Madagascar rosy periwinkle.[1] India has 10,000 native medicinal plants and at least 200 species are estimated for treatment of cancer.[2] About

three-quarters of the world population have used traditional medicine for their health care.[3] Plants have been an important part of sophisticated traditional medicine systems for thousands of years.[4] Furthermore, 50% of anticancer drugs used in clinical trials have been isolated from natural sources (mostly plants) or are related to them.[5] For examples, The Quince Aqueous Fermented Extract (QAFE) obtained from (*Cydonia oblonga*) effectively scavenged the radical target species exhibiting LD_{50} values equal to 68.8l g/mL and 73.7l g/mL towards DPPH and superoxide anion free radicals respectively.[6] Alpinum isoflavone and 40 methoxy licoflavanone are metabolites isolated from *Erythrina suberosa*. They were reported as potent cytotoxic agent towards human leukemia.[7] Aqueous extracts of *Dracaena draco* L exhibited anti proliferation activity in colon and renal cancer.[8] Therefore, traditional medicinal plants due to the bioactive compounds such as flavonoids, phenolic compounds, sterol and glucosinolates can be used as potential sources in the development of new anticancer agents for tumour remedy.[6] *Ficus heterophylla* and *Artocarpus lakoocha* belong to family *Moraceae*. *Ficus heterophylla* is used in colic pain, asthma and dysentery. [9] *Artocarpus lakoocha* is used in anti-herpes simplex virus activity and anti-HIV activity against a wild-type human immuno deficiency virus type 1.[10] *Opuntia dillenii* belong to family *Cactus*. It is used as laxative, stomachic, carminative, antipyretic.[10] Keeping above opinions, the present investigation was taken up to evaluate the phytochemical analysis and cytotoxic screening of petroleum ether, chloroform and methanol extracts of the root powder of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dillenii*.

MATERIALS AND METHOD

Plant material and Preparation of extracts

The roots of *Ficus heterophylla* (*Moraceae*) and *Opuntia dillenii* (*Cactus)* were collected from Cauvery at Sangam near Kanakapura, Ramanagaram district of Karnataka, India and The root of *Artocarpus lakoocha* (*Moraceae*) was collected from Western Ghats area known as Kukku Jatka near Sulya dakshin kannada district of Karnataka, India. All the three drug specimens were identified and authenticated by National Ayurveda Dietetics Research Institute (N.A.D.R.I) Bangaluru. The specimens of all the three drugs were deposited in (N.A.D.R.I) herbarium. The roots were shade dried at room temperature until they were free from moisture. The dried material is reduces to coarse powder in mechanical grinder and passed through 40# sieve to obtain a powder of desire particle size. The uniform powder was subjected to evolution for different parameters. About 500 gm of each root powder was extracted successively with petroleum ether, chloroform and methanol at a temperature 40-45oC to about 40 cycles per batch for 12 batches. The extraction was continued until the solvent in the thimble became clear then few drops of solvent were collected in a test tube during the completion of cycle (during siphoning) and chemical test of that solvent was performed. Extraction was completed only when chemical test shows negative results.

After each extraction the solvent was distilled off in rotavapor and the extract was concentrated at low temperature.

Macroscopically evaluation

The roots powder obtained from above procedure that was subjected to macroscopically evaluation and the results were shown.

Determination of total ash

The roots powder (2g) was placed in silica crucible, previously ignited and weighed. Incinerated by gradually increasing the heat (not exceeding 450^{0C}) until it was free from carbon, crucible was cooled and weighed. Calculated the percentage of ash with reference to the air dried drug.[11]

$$\text{Ash \%} = \frac{\text{Loss in weight}}{W} \times 100$$

Determination of acid-insoluble ash

Boiled the ash for 10 minutes with 25 ml of dilute hydrochloric acid. Collect the insoluble matter in a dry crucible or ashless filter paper, washed with hot water, ignite and weighed. Calculated the percentage of acid- insoluble ash with reference to the air dried drug.[11]

$$\text{Ash \%} = \frac{\text{Loss in weight}}{W} \times 100$$

Determination of water soluble ash

Boiled the ash for 10 minutes with 25 ml of water, collected the insoluble matter in a dry crucible, washed with hot water and ignite to constant weight at a low temperature. Subtracted the weight of insoluble matter from the weight of ash, difference in the weight represents the water-soluble ash. Calculated the percentage of water-soluble ash with reference to air dried drug.[11]

$$\text{Ash \%} = \frac{\text{Loss in weight}}{W} \times 100$$

Determination of alcohol soluble extractive

Macerate 5 gm of the air dried drug coarsely powdered with 100 ml of alcohol in a conical flask for 24 hours, shake frequently during six hours and allow standing for 18 hours. Filtered rapidly taking precaution against loss of alcohol, evaporated 25% of filtrate to dryness in tarred bottomed shallow dish dried at 105^{oC}, weighed and calculated the percentage of alcohol soluble extractive with reference to the air dried drug.[11]

Determination of water soluble extractive

Macerate 5 gm of the air dried drug coarsely powdered with 100ml of chloroform water I.P in a closed flask for 24 hours, shake frequently during six hours and allow standing for 18 hours. Filtered rapidly taking precaution against loss of chloroform, evaporated 25 ml of filtrate to dryness in tarred flat-bottomed shallow dish dried at 105^{oC}, and weighed calculated the percentage of alcohol soluble extractive with reference to the air dried drug.[11]

Loss on drying

About 5 gm of drug was accurately weighed in a petri dish and kept in a hot air oven maintained at 110^{oC}. After cooling in desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.[11]

$$\text{Loss on drying\%} = \frac{\text{Loss in weight}}{W} \times 100$$

Fluorescence analysis of the drugs

Many crude drugs shows the fluorescence when the sample is exposed to ultraviolet radiations. Evaluation of crude drugs based on fluorescence in day light is not much

used, as it is unreliable due to weakness of the fluorescent effect. Fluorescence lamps are fitted with suitable filters, which eliminate visible radiations from the lamp and transmit ultraviolet radiations of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.[11]

Determination of total solid contents

About 5gms of extract was accurately weighed in a petri dish and kept in a hot air oven maintained at 110^{oC} for four hours. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained. Found out total solid contents.[11]

Chemical evaluation of *Ficus heterophylla, Artocarpus lakoocha* and *Opuntia dillenii* roots powder

Chemical analysis of roots powder was performed using different chemical reagents like picric acid, concentrated H_2SO_4, aqueous ferric chloride, iodine solution, ammonia solution, aqueous potassium hydroxide, aqueous mercuric-di- hydride, aqueous silver nitrate and magnesium hydrochloride to detect the phytoconstituents with colour change under ordinary day light by the standard method. [11]

Phytochemical screening:

The petroleum ether, chloroform and methanol roots extracts of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dillenii* were subjected to phytochemical screening according to the phytochemical standard methods of Raman N, 2006.[12]

Cytotoxic screening by *Allium cepa* root model

Healthy and equal sized bulbs of common onion (*Allium cepa*) were chosen and the loose outer scales of bulbs and old roots were removed with the help of sharp and pointed forceps so as to expose the root primordial series of bulbs were grown in different concentration of extracts (0.2, 0.4, 0.6 and 0.8 mg/ml) in beakers at 27 ± 2 $^{\circ C}$ in a dark cupboard. At end of exposure periods (48 hours), root lengths were measured in cm with ruler .The onions were grown on distilled water (without any extract) serve as the control. Roots were excised at 48 hr and suspended in fixative liquid (ethanol:acetic acid (3:1 v/v)) for 24 hrs. They were washed with distilled water, hydrolyzed at 60^{oC} in 1N HCl for 1 min and transferred to watch glass containing aceto-orcien and 1N HCl (9:1). They were then heated intermittently for 5-10 min, covered and kept aside for 20-30 min. The tip of root was cut with sharp blade and placed on a glass slide in a drop of 45% glacial acetic acid and covered with coverslip. The root tip was squashed by tapping with match stick and sealed with DPX (Distyrene a plasticizer and xylene). The cells were scored under high power objective in the compound microscope for mitotic index (% MI).[13]

$$\% \text{ MI} = \frac{\text{Total number of cells in mitosis}}{\text{Total number of cells counted}} \times 100$$

Statistical analysis

All data were recorded as mean ± standard deviation of triplicate measurements. Significant differences among treatment

means were determined by ANOVA at P<0.05.

RESULTS & DISCUSSION

Macroscopically evaluation

Macroscopically evaluation revealed that *Ficus heterophylla* roots powder is yellowish, with bitter taste, characteristic odour and smooth fracture. *Artocarpus lakoocha* roots powder is brownish with bitter taste, characteristic odour and smooth fracture, *Opuntia dillenii* roots powder is pale yellowish with bitter taste, characteristic odour and rough fracture.

Ash values

Ash values means total ash, acid insoluble ash and water insoluble ash, so ash values of all the three drugs were reported in table 1.

Table 1: Ash values of *Ficus heterophylla, Artocarpus lakoocha,*and *Opuntia dillenii* roots powders

Parameters	*Ficus heterophylla*	*Artocarpus lakoocha*	*Opuntia dillenii*
Total ash	4.852 % w/w	4.652 % w/w	4.352 % w/w
Acid insoluble ash	2.514 % w/w	2.453 % w/w	2.182 % w/w
Water insoluble ash	3.182 % w/w	2.852 % w/w	2.735 % w/w

Extractive values

Extractive values means alcohol soluble extractives and water soluble extractives, so extractive values were reported in table 2.

Table 2: Extractive values of *Ficus heterophylla, Artocarpus lakoocha,*and *Opuntia dillenii* roots powders

Parameters	*Ficus heterophylla*	*Artocarpus lakoocha*	*Opuntia dillenii*
Alcohol soluble extractive	26.31 % w/w	23.56 % w/w	25.65 % w/w
Water soluble extractive	13.56 5% w/w	12.25 % w/w	13.46 % w/w

Loss on drying

Ficus heterophylla, Artocarpus lakoocha and *Opuntia dillenii* roots powders have shown the loss on drying 5.65 % w/w, 5.76 % w/w and 8.25 % w/w respectively.

Fluorescence analysis of the drug

Ficus heterophylla, Artocarpus lakoocha and *Opuntia dillenii* roots powders have revealed the yellowish blue fluorescence, yellowish brown fluorescence and yellowish green fluorescence respectively.

Total solid contents

The petroleum ether, chloroform and methanol extracts of *Ficus heterophylla*

roots powders displayed total solid contents 72.52% w/w, 83.57 % w/w and 85.55% w/w respectively. The petroleum ether, chloroform and methanol extracts of *Artocarpus lakoocha* roots powders exhibited total solid contents 75.62% w/w, 81.56 % w/w and 85.65% w/w respectively. The petroleum ether, chloroform and methanol extracts of *Opuntia dillenii* roots powders manifested total solid contents 75.62% w/w, 82.77 % w/w and 84.25% w/w respectively.

Chemical evaluation of *Ficus heterophylla, Artocarpus lakoocha* and *Opuntia dillenii* roots powder

Table 3 shows the chemical evaluation of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dillenii* roots powder for the presence of alkaloids, tannins and flavonoids.

Table 3: Chemical evaluation of *Ficus heterophylla, Artocarpus lakoocha* and *Opuntia dillenii* roots powder.

Reagents	*Ficus heterophylla*		*Artocarpus lakoocha*		*Opuntia dillenii*	
	Color / ppt	**Chemical constituent**	**Color/ ppt**	**Chemical constituent**	**Color/ ppt**	**Chemical constituent**
Picric acid	yellow	Alkaloids present	yellow	Alkaloids present	yellow	Alkaloids present
Conc.H_2SO_4	No Reddish brown color	Steroids, Lipids absent	No Reddish brown	Steroids, Lipids absent	No Reddish brown	Steroids, Lipids absent
Aq. $FeCl_3$	Blue/black color	Hydrosoluble tannins	Blue/black color	Hydrosoluble tannins	Blue/black color	Hydrosoluble tannins
Iodine solution	No blue color	Starch absent	No blue color	Starch absent	No blue color	Starch absent
Ammonia solution	No change	Aanthraqui-one glycol-sides absent	No change	Aanthraqui-none glyco -sides absent	No change	Aanthraqui-none glyco -sides absent
5%Aq.KOH	No change	Aanthraqui-none glyco-sides absent	No change	Aanthraqui-none glyco-sides absent	No change	Aanthraqui-none glyco-sides absent
Aq. HgH_2	Precipitate	Alkaloids present	Precipitate	Alkaloids present	Precipitate	Alkaloids present
Aq. $AgNO_3$	No Precipitate	Proteins absent	No Precipitate	Proteins absent	No Precipitate	Proteins absent
Mg-Hcl	Yellow color	Flavonoids present	Yellow color	Flavonoids present	Yellow color	Flavonoids present

Qualitative phytochemical screening of *Ficus heterophylla, Artocarpus lakoocha* and *Opuntia dillenii* roots powder

Table 4.1, 4.2 and 4.3 show phytochemical compounds which are present in petroleum ether, chloroform and methanol extracts of *Ficus heterophylla, Artocarpus lakoocha* and *Opuntia dillenii* roots powder respectively. The petroleum ether extract of *Ficus heterophylla* and *Opuntia dillenii* contained carbohydrates, gums, sterols, terpenoids, fats and oil. The petroleum ether extract of *Artocarpus lakoocha* contained carbohydrates, gums, and sterols. Chloroform and methanol extracts of all three plants contained carbohydrates, gums, fats and oils sterols, alkaloids, terpenoids, flavonoids, tannins and phenolic compounds

Table 4.1: Qualitative phytochemical screening of petroleum ether, chloroform and methanol extracts of *Ficus heterophylla*

Name of the plant				*Ficus heterophylla* roots extracts		
Sl.No	**Name of chemical test**			**Petroleum ether extract**	**Chloroform extract**	**Methanol extract**
5.6.1	**Tests for carbohydrates**			+	+	+
	Molish's test (General test)					
	A	**Tests for reducing sugars**				
		a)	**Fehling's test**	–	–	+
		b)	**Benedict's test**	–	–	+
	B	**Test for mono saccharides Barfoed's test**		–	–	–
	C	**Test for pentose sugar**		–	–	–
	D	**Test for hexose sugars**				
		a)	**Tollens ploroglucin-ol test for galactose**	–	–	+
		b)	**Cobalt chloride test**	–	–	Fructose
	E	**Test for non-reducing sugars**		–	–	–
	F	**Test for non-reducing poly-saccharides (Starch)**				
		a)	**Iodine test**	–	–	–
		b)	**Tannic acid test for starch**	–	–	–
	G	**Test for gums**		+	+	+
5.6.2	**Test for proteins**					

	a)	**Biuret test (General test)**	–	–	–
	b)	**Millon's test (for protein)**	–	–	–
	c)	**Xanthoprotein test (for protein containig tyrosine or tryptophan)**	–	–	–
	d)	**Test for proteins containing sulphur**	–	–	–
	e)	**Precipitation test**	–	–	–
5.6.3	**Tests for amino acids**				
	a)	**Ninhydrin test (General test)**	–	–	–
	b)	**Test for tyrosine**	–	–	–
	c)	**Test for tryptophan**	–	–	–
	d)	**Test for cysteine**	–	–	–
5.6.4	**Tests for fats and oils**				
	a)	**Solubility test**	+	+	–
	b)	**Saponification test**	+	+	–
	c)	**Filter paper test**	+	+	–
5.6.5	**Tests for sterols**				
	a)	**Salkowski reaction**	+	+	–
	b)	**Libermann-Bourchard reaction**	+	+	–
	c)	**Libermann's reaction**	+	+	–
5.6.6	**Tests for triterpenoids**				
	a)	**Salkowski reaction**	–	+	+
	b)	**Libermann-Bourchard reaction**	–	+	+
5.6.7	**Tests for glycosides**				
	A	**Tests for cardiac - glycosides**			
		a) **Baljet's test**	–	–	–
		b) **Legal's test (Test for cardenoloids)**	–	–	–
		c) **Keller killianic test (Test for deoxy sugar)**	–	–	–

		d)	**Libermann's test (Test for bufadinolides)**	–	–	–
	B	**Test for anthraquinone glycosides**				
		a)	**Borntrager's test for anthraquinone glycosides**	–	–	–
		b)	**Modified Borntr--ager's test for C- glycosides**	–	–	–
	C	**Tests for saponin glycosides**				
		a)	**Foam test**	–	–	–
		b)	**Heamolytic test**	–	–	–
	D	**Tests for cyanogenetic glycosides (Guinord reaction or sodium test)**		–	–	–
	E	**Tests for coumarin glycosides**				
		a)	**Alkaline reagent test**	–	–	–
		b)	**NaOH Soaked paper test**	–	–	–
5.6.8	**Test for flavonoids**					
	a)	**Ferric chloride test**		–	+	+
	b)	**Shinoda test**		–	+	+
	c)	**Alkaline reagent test**		–	+	+
	d)	**Lead acetate solution test**		–	+	+
5.6.9	**Tests for alkaloids**					
	a)	**Dragendroff's test**		–	+	+
	b)	**Mayer's test**		–	+	+
	c)	**Hager's test**		–	+	+
	d)	**Wagner's test**		–	+	+
	e)	**Murexide test for purine alkaloids**		–	–	–
5.6.10	**Tests for tannins and phenolic compounds**					

	a)	5% $FeCl_3$ solution	+	–	+
	b)	Lead acetate solution	+	–	+
	c)	Gelatin solution	+	–	+
	d)	Bromine water	+	–	+
	e)	Acetic acid solution	+	–	+
	f)	Dilute iodine solution	+	–	+
	g)	Dilute HNO_3	+	–	+
	h)	Dilute potassiumperma-ngnate solution	+	–	+
5.6.11	Tests for saponins				
	a)	Foam test	–	–	–
	b)	Raymond's test	–	–	–
	c)	Haeolysis test	–	–	–
	d)	Bromine water test	–	–	–
	e)	Legal's test	–	–	–
5.6.12	Test for lipids		–	–	–

Table 4.2: Qualitative phytochemical screening of petroleum ether, chloroform and methanol extracts of *Artocarpus lakoocha*

Name of the plant			*Artocarpus lakoocha* roots extracts		
Sl.No	Name of chemical test		Petroleum ether extract	chloroform extract	methanol extract
5.6.1	Tests for carbohydrates		+	+	+
	Molish's test (General test)				
	A	Tests for reducing sugars			
		a) Fehling's test	–	+	+
		b) Benedict's test	–	+	+
	B	Test for mono saccharides Barfoed's test	–	–	–
	C	Test for pentose sugar	–	–	–
	D	Test for hexose sugars			
		a) Tollens ploroglucin--ol test for galactose	–	+	+

		b) Cobalt chloride test	–	Glucose	Glucose
	E	**Test for non-reducing sugars**	–	–	–
	F	**Test for non-reducing poly-saccharides (Starch)**			
		a) Iodine test	–	–	–
		b) Tannic acid test for starch	–	–	–
	G	**Test for gums**	+	+	+
5.6.2		**Test for proteins**			
	a)	**Biuret test (General test)**	–	–	–
	b)	**Millon's test (for protein)**	–	–	–
	c)	**Xanthoprotein test (for protein containig tyrosine or tryptophan)**	–	–	–
	d)	**Test for proteins containing sulphur**	–	–	–
	e)	**Precipitation test**	–	–	–
5.6.3		**Tests for amino acids**			
	a)	**Ninhydrin test (General test)**	–	–	–
	b)	**Test for tyrosine**	–	–	–
	c)	**Test for tryptophan**	–	–	–
	d)	**Test for cysteine**	–	–	–
5.6.4		**Tests for fats and oils**			
	a)	**Solubility test**	–	+	+
	b)	**Saponification test**	–	+	+
	c)	**Filter paper test**	–	+	+
5.6.5		**Tests for sterols**			
	a)	**Salkowski reaction**	+	–	+
	b)	**Libermann-Bourchard reaction**	+	–	+
	c)	**Libermann's reaction**	+	–	+
5.6.6		**Tests for triterpenoids**			
	a)	**Salkowski reaction**	+	+	–
	b)	**Libermann-Bourchard reaction**	+	+	–
5.6.7		**Tests for glycosides**			
	A	**Tests for cardiac glycosides**			

		a)	**Baljet's test**	–	–	–
		b)	**Legal's test (Test for cardenoloids)**	–	–	–
		c)	**Keller killianic test (Test for deoxy sugar)**	–	–	–
		d)	**Libermann's test (Test for bufadinolides)**	–	–	–
	B	**Test for anthraquinone glycosides**				
		a)	**Borntrager's test foranthraquinone glycosides**	–	–	–
		b)	**Modified Borntr--ager's test for C- glycosides**	–	–	–
	C	**Tests for Saponin glycosides**				
		a)	**Foam test**	–	–	–
		b)	**Heamolytic test**	–	–	–
	D	**Tests for cyanogenetic glycosides (Guinord reaction or sodium test)**		–	–	–
	E	**Tests for coumarin glycosides**				
		a)	**Alkaline reagent test**	–	–	–
		b)	**NaOH Soaked paper test**	–	–	–
5.6.8	**Test for flavonoids**					
	a)	**Ferric chloride test**		–	+	+
	b)	**Shinoda test**		–	+	+
	c)	**Alkaline reagent test**		–	+	+
	d)	**Lead acetate solution test**		–	+	+
5.6.9	**Tests for alkaloids**					
	a)	**Dragendroff's test**		–	+	+
	b)	**Mayer's test**		–	+	+
	c)	**Hager's test**		–	+	+
	d)	**Wagner's test**		–	+	+
	e)	**Murexide test for purine alkaloids**		–	–	–
5.6.10	**Tests for tannins and phenolic compounds**					
	a)	**5% $FeCl_3$ solution**		–	+	+
	b)	**Lead acetate solution**		–	+	+

	c)	Gelatin solution	–	+	+
	d)	Bromine water	–	+	+
	e)	Acetic acid solution	–	+	+
	f)	Dilute Iodine solution	–	+	+
	g)	Dilute HNO_3		+	+
	h)	Dilute Potassiumperma--ngnate solution	–	+	+
5.6.11	Tests for saponins		–	–	–
	a)	Foam test	–	–	–
	b)	Raymond's test	–	–	–
	c)	Haemolysis test	–	–	–
	d)	Bromine water test	–	–	–
	e)	Legal's test	–	–	–
5.6.12	Test for lipids		–	–	–

Table 4.3: Qualitative phytochemical screening of petroleum ether, chloroform and methanol extracts of *Opuntia dilleni*

Name of the plant			*Opuntia dillenii* roots extracts		
Sl.No	Name of chemical test		Petroleum ether extract	Chloroform extract	Methanol extract
5.6.1	Tests for carbohydrates		+	+	+
	Molish's test (General test)				
	A	Tests for reducing sugars			
		a) Fehling's test	–	+	+
		b) Benedict's test	–	+	+
	B	Test for mono saccharides Barfoed's test	–	–	–
	C	Test for pentose sugar	–	–	–
	D	Test for hexose sugars			
		a) Tollens ploroglucin-ol test for galactose	–	+	+
		b) Cobalt chloride test	–	Glucose	Glucose
	E	Test for non-reducing sugars	–	–	–
	F	Test for non-reducing poly- saccharides (Starch)			
		a) Iodine test	–	–	–
		b) Tannic acid test for starch	–	–	–

	G	Test for gums	+	+	+
5.6.2		Test for proteins			
	a)	Biuret test (General test)	–	–	–
	b)	Millon's test (for protein)	–	–	–
	c)	Xanthoprotein test (for protein containig tyrosine or tryptophan)	–	–	–
	d)	Test for Proteins containing sulphur	–	–	–
	e)	Precipitation test	–	–	–
5.6.3		Tests for amino acids			
	a)	Ninhydrin test (General test)	–	–	–
	b)	Test for tyrosine	–	–	–
	c)	Test for tryptophan	–	–	–
	d)	Test for cysteine	–	–	–
5.6.4		Tests for fats and oils			
	a)	Solubility test	–	+	+
	b)	Saponification test	–	+	+
	c)	Filter paper test	–	+	+
5.6.5		Tests for sterols			
	a)	Salkowski reaction	–	+	+
	b)	Libermann-Bourchard reaction	–	+	+
	c)	Libermann's reaction	–	+	+
5.6.6		Tests for triterpenoids			
	a)	Salkowski reaction	+	+	–
	b)	Libermann-Bourchard reaction	+	+	–
5.6.7		Tests for glycosides			
	A	Tests for cardiac glycosides			
		a) Baljet's test	–	–	–
		b) Legal's test (Test for cardenoloids)	–	–	–
		c) Keller killianic test (Test for deoxy sugar)	–	–	–
		d) Libermann's test (Test for bufadinolides)	–	–	–
	B	Test for anthraquinone glycosides			

		a)	Borntrager's test for anthraquinone glycosides	–	–	–
		b)	Modified Borntr--ager's test for C- glycosides	–	–	–
	C	Tests for Saponin glycosides				
		a)	Foam test	–	–	–
		b)	Heamolytic test	–	–	–
	D	Tests for cyanogenetic glycosides (Guinord reaction or sodium test)		–	–	–
	E	Tests for coumarin glycosides				
		a)	Alkaline reagent test	–	–	–
		b)	NaOH Soaked paper test	–	–	–
5.6.8	Test for flavonoids					
	a)	Ferric chloride test		–	+	+
	b)	Shinoda test		–	+	+
	c)	Alkaline reagent test		–	+	+
	d)	Lead acetate solution test		–	+	+
5.6.9	Tests for alkaloids					
	a)	Dragendroff's test		–	+	+
	b)	Mayer's test		–	+	+
	c)	Hager's test		–	+	+
	d)	Wagner's test		–	+	+
	e)	Murexide test for purine alkaloids		–	–	–
5.6.10	Tests for tannins and phenolic compounds					
	a)	5% $FeCl_3$ solution		–	+	+
	b)	Lead acetate solution		–	+	+
	c)	Gelatin solution		–	+	+
	d)	Bromine water		–	+	+
	e)	Acetic acid solution		–	+	+
	f)	Dilute Iodine solution		–	+	+
	g)	Dilute HNO_3		–	+	+
	h)	Dilute potassiumperma--ngnate solution		–	+	+
5.6.11	Tests for saponins					

	a)	**Foam test**	–	–	–
	b)	**Raymond's test**	–	–	–
	c)	**Haemolysis test**	–	–	–
	d)	**Bromine water test**	–	–	–
	e)	**Legal's test**	–	–	–
5.6.12	**Test for lipids**		–	–	–

Cytotoxic screening by *Allium cepa* root model

Table 5.1, 5.2 and 5.3 show effect of petroleum ether, chloroform and methanol extracts of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dillenii* roots powder on root length and mitotic index of *Allium cepa* respectively. The petroleum ether, chloroform and methanol extracts of *Ficus heterophylla* showed significant root growth inhibition in *A. cepa* at concentrations of 800 µg/ml induced root growth inhibitions of 2.15, 1.09 and 0.89 cm respectively at 72h. The petroleum ether, chloroform and methanol extracts of *Artocarpus lakoocha* indicated significant root growth inhibition in *A. cepa* at concentrations of 800 µg/ml induced root growth inhibitions of 0.75, 0.89 and 0.89 cm respectively at 72h. The 800 µg/ml of methanol extracts of *Ficus heterophylla* and chloroform and methanol of *Artocarpus lakoocha* displayed 35.49% reduction in mitotic index after 72h. The petroleum ether, chloroform and methanol extracts of *Opuntia dillenii* showed significant root growth inhibition in *A. cepa* at concentrations of 800µg/ml induced root growth inhibitions of 1.75, 1.09 and 0.99 cm respectively at 72h. The 800 µg/ml of methanol extracts of *Opuntia dillenii* indicated 39.56% reduction in mitotic index after 72h. The methanol extracts of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dillenii* roots powder induced chromosomal aberrations include; anaphase bridge, multiple bridge, anaphase bridge and fragments, criss-cross at anaphase, telophase with vagrant and polar deviation

Table 5.1: Effect of different extracts of *Ficus heterophylla* on root length and mitotic index of *Allium cepas*

Name of the extract	48 hrs		72 hrs	
	Root length (in cm)	% MI	Root length (in cm)	% MI
Control (Distilled water)	1.87 ± 0.35	72.68	2.82 ± 0.92	69.72
Ficus heterophylla petroleum ether extract				
0.2mg (200 µg) /ml	1.86 ± 0.61[a]	71.42	2.72 ± 0.64[a]	68.59
0.4mg(400 µg) /ml	1.84 ± 0.91[a]	71.36	2.66 ±	67.86

			0.50[a]	
0.6mg (600 µg)/ml	1..83 ± 0.24[a]	70.85	2.39 ± 0.15[a]	67.34
0.8mg (800 µg) /ml	1.81 ± 0.29[a]	70.56.6	2.15 ± 0.16[b]	67..13
Ficus heterophylla chloroform extract				
0.2mg (200 µg) /ml	1.88 ± 0.35[a]	67.92	1.86 ± 0.33[a]	66.02
0.4mg(400 µg) /ml	1.76 ± 0.2[a]	66.02	1.75 ± 0.78[a]	64.61
0.6mg (600 µg)/ml	1..42 ± 0.29[a]	56.12	1.32 ± 0.06[a]	59.72
0.8mg (800 µg) /ml	1.05 ± 0.28[b]	34.62	1.09 ± 0.25[b]	45.49
Ficus heterophylla methanol extract				
0.2mg (200 µg) /ml	1.88 ± 0.35[a]	67.92	1.86 ± 0.33[a]	66.02
0.4mg(400 µg) /ml	1.40 ± 0.2[a]	64.02	1.25 ± 0.78[a]	60.61
0.6mg (600 µg)/ml	0.92 ± 0.29[a]	56.12	1.02 ± 0.06[b]	58.72
0.8mg (800 µg) /ml	0.75 ± 0.28[b]	34.62	0.89 ± 0.25[c]	35.49

n=3, Values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. P values: a< 0.05, b<0.01, c< 0.001, compared to the control group

Table 5.2: Effect of different extracts of *Artocarpus lakoocha* on root length and mitotic index of *Allium cepas*

Name of the extract	48 hrs		72 hrs	
	Root length (in cm)	% MI	Root length (in cm)	% MI
Control	1.87 ± 0.35	72.68	2.82 ± 0.92	69.72
Artocarpus lakoocha petroleum ether extract				
0.2mg (200 µg) /ml	1.82 ± 0.61[a]	70.42	2.32 ± 0.64[a]	67.59

0.4mg(400 µg) /ml	1.55 ± 0.91[a]	65.02	1.96 ± 0.50[a]	60.36
0.6mg (600 µg)/ml	0.97 ± 0.24[a]	50.18	1.09 ± 0.15[a]	58.04
0.8mg (800 µg) /ml	0.62 ± 0.29[a]	37.62	0.75 ± 0.16[a]	23.63
Artocarpus lakoocha chloroform extract				
0.2mg (200 µg) /ml	1.88 ± 0.35[a]	67.92	1.86 ± 0.33[b]	66.02
0.4mg(400 µg) /ml	1.40 ± 0.2[b]	64.02	1.25 ± 0.78[b]	60.61
0.6mg (600 µg)/ml	0.92 ± 0.29[b]	56.12	1.02 ± 0.06[b]	58.72
0.8mg (800 µg) /ml	0.75 ± 0.28[b]	34.62	0.89 ± 0.25[b]	35.49
Artocarpus lakoocha methanol extract				
0.2mg (200 µg) /ml	1.88 ± 0.35[b]	67.92	1.86 ± 0.33[b]	66.02
0.4mg(400 µg) /ml	1.40 ± 0.2[b]	64.02	1.25 ± 0.78[c]	60.61
0.6mg (600 µg)/ml	0.92 ± 0.29[b]	56.12	1.02 ± 0.06[c]	58.72
0.8mg (800 µg) /ml	0.75 ± 0.28[c]	34.62	0.89 ± 0.25[c]	35.49

n=3, Values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. P values: a< 0.05, b<0.01, c< 0.001, compared to the control group

Table 5.3: Effect of different extracts of *Opuntia dillenii* on root length and mitotic index of *Allium cepas*

Name of the extract	48 hrs		72 hrs	
	Root length (in cm)	% MI	Root length (in cm)	% MI
Control	1.87 ± 0.35	72.68	2.82 ± 0.92	69.72
Opuntia dillenii petroleum ether extract				
0.2mg (200 µg) /ml	1.83 ± 0.61[a]	71.42	2.52 ± 0.64[a]	68.59
0.4mg(400 µg) /ml	1.75 ± 0.91[a]	69.02	2.26 ± 0.50[b]	67.36

0.6mg (600 µg) /ml	1.57 ± 0.24[a]	50.18	1.90 ± 0.15[b]	65.04
0.8mg (800 µg) /ml	1.32 ± 0.29[a]	47.62	1.75 ± 0.16[b]	63.63
Opuntia dillenii chloroform extract				
0.2mg (200 µg) /ml	1.88 ± 0.35[b]	67.92	1.86 ± 0.33[c]	66.02
0.4mg(400 µg) /ml	1.50 ± 0.2[c]	65.02	1.35 ± 0.78[c]	62.61
0.6mg (600 µg)/ml	1.12 ± 0.29[c]	63.12	1.12 ± 0.06[c]	58.72
0.8mg (800 µg) /ml	0.85 ± 0.28[c]	55.62	1.09 ± 0.25[c]	45.49
Opuntia dillenii methanol extract				
0.2mg (200 µg) /ml	1.88 ± 0.35[c]	67.92	1.86 ± 0.33[c]	66.02
0.4mg(400 µg) /ml	1.41 ± 0.2[c]	63.02	1.25 ± 0.78[c]	62.61
0.6mg (600 µg)/ml	0.91 ± 0.29[c]	54.12	1.01 ± 0.06[c]	59.72
0.8mg (800 µg) /ml	0.79 ± 0.28[c]	36.62	0.99 ± 0.25[c]	39.56

n=3, Values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. P values: a< 0.05, b<0.01, c< 0.001, compared to the control group

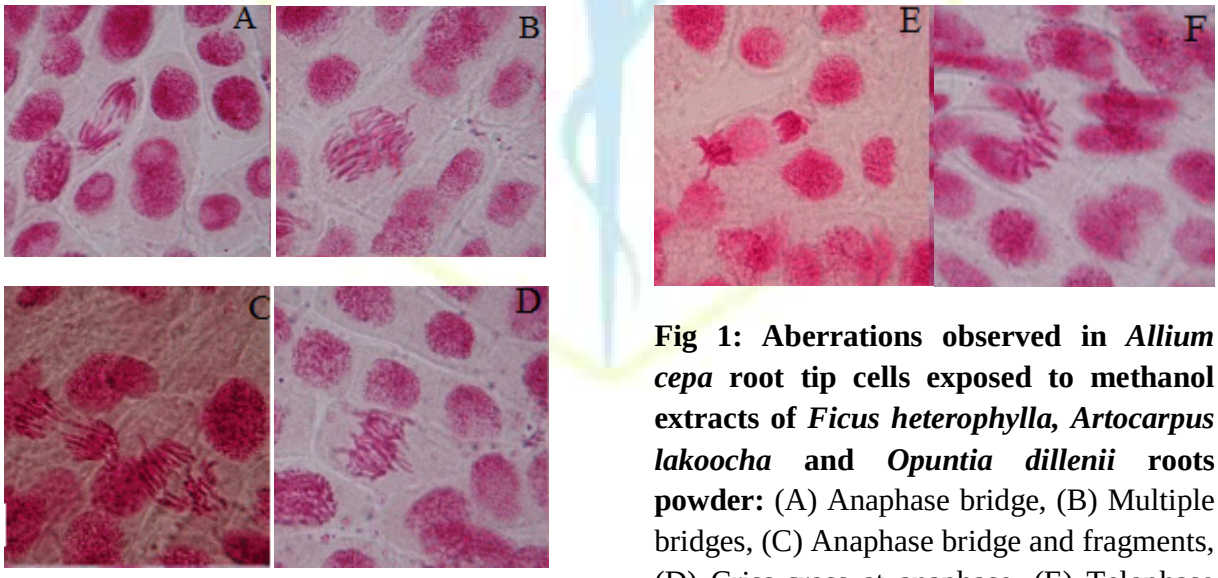

Fig 1: Aberrations observed in *Allium cepa* root tip cells exposed to methanol extracts of *Ficus heterophylla, Artocarpus lakoocha* and *Opuntia dillenii* roots powder: (A) Anaphase bridge, (B) Multiple bridges, (C) Anaphase bridge and fragments, (D) Criss-cross at anaphase, (E) Telophase with vagrant chromosome and (F) Polar deviation.

The incidence of cancer has gradually been raised over the last decades. A substantial number of patients have to go through chemotherapy and radiotherapy. These remedies can induce severe side effects like bone marrow depression such as erythrocytes, thrombocytes and neutrophils, weight loss, nausea, vomiting, diarrhoea and low appetite. Conventional medical treatments can avert these side effects sufficiently. Using Ayurveda herbal treatments to remedy these side effects is a new approach, which is an effort to help those patients whose side effects can't be sufficiently prevented or treated by modern medicine.[14] Ayurveda, a traditional Indian medicine of plant drugs has been successful recently for preventing or suppressing various tumours. Each herbal contains multiple active compounds that can induce synergistically, producing remedial benefits and diminishing the adverse effects.[15] Survey of literature revealed that various parts of plants such as roots, leaves, bark and flowers which were found to be used in Ayurveda system of medicine for cancer therapy. [15] Data of current research exhibited the methanol extract of roots powder of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dilleni* have significant cytotoxic effect by reason of containing of bioactive phytochemical constituents like alkaloids, phenolic compounds and flavonoids which their role as natural antioxidants and free radical scavenging have been proved already.[16]

CONCLUSION

Methanol extracts of roots powder of *Ficus heterophylla*, *Artocarpus lakoocha* and *Opuntia dilleni* displayed superior cytotoxic effect because of having the inhibitory effect on the growth of *Allium cepa* root meristem and decreasing the mitotic index percentage. The cytotoxic activity raised with increase in concentration and time of exposure to the extracts. The present investigation showed the biologically active phytochemicals such as flavonoids, tannins, phenolic compounds which can be beneficial for remedy of different illness. Hence, it can be appraised useful for supportive cancer therapy.

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