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In-Vitro Cytotoxic Activity of *Persea Americana* Miller Leaves Extracts

Narayan R Miskin*[1], Manjunatha S Katagi[1], Girish Bolakatti[2], Vishvakarma kamala Sunita[1], Suchitra.M[1]

[1]*Bapuji Pharmacy College, SS Layout, Davangere-577004, Karnataka, India.*
[2]*GM Institute of Pharmaceutical Sciences and Research, Davangere-577006, Karnataka, India.*

A B S T R A C T
Phytochemistry plays an important role for curing of various human diseases with slight side effects. Such medicinal plants have bioactive compounds may be primary or secondary metabolites. The present study aims to evaluate that the In-vitro anticancer activity of *Persea americana* miller leaves extracts in different cancer cell lines. Dried Coarse powered leaves of *Persea americana* Miller was successively extracted with various solvents based on their polarity such as petroleum ether, chloroform, ethyl acetate and aqueous extract by maceration. Qualitative phytochemical screening was performed to detect phytochemical constituents of the extracts. Cytotoxic activity of plant leaves extracts on MCF-7 and HeLa cells was investigated *in vitro* through MTT bioassay. All the extracts except EAPA has showed IC50 value of 233.7µg/ml, 182.0µg/ml, 203.7µg/ml, 147.7µg/ml, 121.0µg/ml respectively in MCF-7 cells. All the extracts have showed IC50 value 200.2µg/ml, 105.4µg/ml, 285.8µg/ml, 132.7µg/ml and 214.4µg/ml respectively in HeLa cells.
Keywords: *Persea americana* miller, cytotoxic activity, MTT assay.

A R T I C L E I N F O

Corresponding Author
Narayan R Miskin
Bapuji Pharmacy College,
SS Layout, Davangere-577004,
Karnataka, India.

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C O N T E N T S

1. Introduction

Cancer is characterized by uncontrolled division of cells and the ability of these cells to invade other tissues either by direct growth into adjacent tissues through invasion or by implantation into distant sites by metastasis[1]. Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries and the burden of cancer is increasing in economically developing countries as a result of[2] population aging and growth as well as increasingly an adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and "westernized" diets[3]. It has been estimated that the total number of new cases of cancer will rise from 10 million in year 2000 by

approximately 25% in each decade, reaching 24 million new cases per year in the year 2050; the total number of deaths will rise from 6 million in the year 2000 to 10 million in 2020 to over 16 million in the year 2050; in the year 2050, there will be 17 million new cases of cancer in less developed countries, while only 7 million new cases of cancer will occur in the more developed countries[4,5,6].

In chemotherapeutic cancer natural or synthetic biological or chemical agents are used to reverse, suppress or prevent carcinogenic progression[5]. Historically natural products have been employed as anticancer agents for quite a considerable period of human existence and through the years have been incorporated into both traditional and allopathic medicine. A significant number of chemotherapeutic drugs in current use were either isolated from plant species or derived from natural prototype[2,6]. The following are some of excellent examples of vinca alkaloids, vincristine, vinbalastine isolated from catharanthus roseus, etoposides and teneposide, the semi synthetic derivative of epipodophylotoxin, isolated from *Podophyllum* species and the naturally derived taxanes isolated from taxus species and semisynthetic deravative of camptothecin, irinotecan and topotecan, isolated from *Camptotheca accuminata*[7,8]. In fact, according to Cragg and Newman, more than 50% of drugs in clinical trials from natural sources or are related to them[9].

Plant materials have a long history of used in the treatment of cancer. Hartwell has reported in his review about the 300 plants species used against[10, 11, 13]. Plant based discovery has resulted in the development of many anticancer drugs currently in clinical use. Besides this it also provides a platform for the design of novel and safe drugs through a proper understanding of the complete synergistic interaction of various constituents of anticancer herbs[14, 15, 16]

Persea americana (family Lauraceae) is a tree plant known as 'avocado', 'avocado pear' or 'alligator pear'. They are widely cultivated throughout the tropics and subtropics of the world for their edible fruits and for some economic and therapeutic uses[17]. Avocado tree is known only for the fruit that people usually consume. Apparently avocado leaf is one of the natural ingredients that can be used as a traditional medicine[18]. This leaf has been empirically used as a diuretic, analgesic, anti-inflammatory, hypertensive, hypoglycemic, diarrhea, sore throat and hemorrhage cure.

Avocado is one of the fruit plant groups which are nutritious as a preservative and antioxidants[19,20]. Avocado flesh can be used as an anti-hyperlipidemia and has antioxidant activity and reduce the risk of metabolic syndrome[21,22,23].One part of the avocado plant that has the potential as a natural antioxidant substance is avocado leaf. Previous research has shown that avocado leaf has the potential as a natural antioxidant[24] and positively contains alkaloids, flavonoids, saponins, tannins and steroids using methanol solution to hydrolyze and extract avocado leaf[25].
The present study was aimed to investigate the *in vitro* cytotoxic potential of Petroleum ether, chloroform, ethyl acetate and aqueous extracts of leaves of *Persea americana* miller against MCF-7 and HeLa cancer cells by MTT bioassay.

2. Material and Methods

Collection of plant material and extraction:

The leaves of plant *Persea americana* Miller used for the present study were collected from the local surroundings of Davangere and authenticated by professor L.C. Kulkarni, Department of botany, P.C.Jabin science college, Hubballi. Authenticated leaves of *Persea americana* Miller. were shade dried and pulverized into coarse material. ≠120gms per batch coarsely dried powered leaves of *Persea americana* Miller were successively extracted with various solvents in increasing order of polarity such as petroleum ether, chloroform, ethyl acetate and macerating with water. Percentage yield of various extracts of *Persea americana* Miller leaves were determined. Preliminary phytochemical analysis is carried out to assess the presence of various phytoconstituents present in plant leaves extracts.

***Invitro* anticancer activity by MTT Bioassay**

Cytotoxicity studies for MCF-7 and HeLa cell lines

Cell lines and culture medium:

All the cell lines were procured from ATCC stock cells was cultured in DMEM/RPMI .The monolayer cell culture was trypsinized and the cell count was adjusted to 5.0 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37^{o}C for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line[30,31].

Calculating Inhibition:

% Inhibition = ((OD of Control – OD of sample)/OD of Control) x 100.

3. Results and Discussion

Successive extraction with different solvents and their % yield was reported in Table no 1 . phytochemical screening of all extracts were done and reported their phytochemical constituents in Table no2. Cytotoxicity activity of four extracts were carried out against MCF 7 cell line and HeLa cell line at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. Results of different concentrations of *Persea americana* Miller including 10 – 320ug/ml are tabulated in Table no 3, and graphically

represented in Figure 1. MTT assay of Persea *americana* Miller shows significant effect on MCF 7 cell line and HeLa cell in concentration range between 10 – 320ug/ml compared with control. The highest cytotoxicity of this extract against MCF 7 cell line and HeLa cell line was found in concentration 121ug/ml and 99.14 ug/ml. It was found that the percentage of growth inhibition to be increasing with increasing concentration of test compounds and IC50 value of PEPA and AQPA are good towards MCF 7 and HELA cell line respectively.

Table: 1 Percentage yield of various extracts of *Persea americana* Miller leaves

Extracts Successive extraction	**% Dry weight in grams**
Petroleum ether (40^0-60^0C) extract (PEPA)	6.4
Chloroform extract(CHPA)	5.09
Ethyl acetate extract(EAPA)	4.0
Aqueous extract(AQPA)	16.4

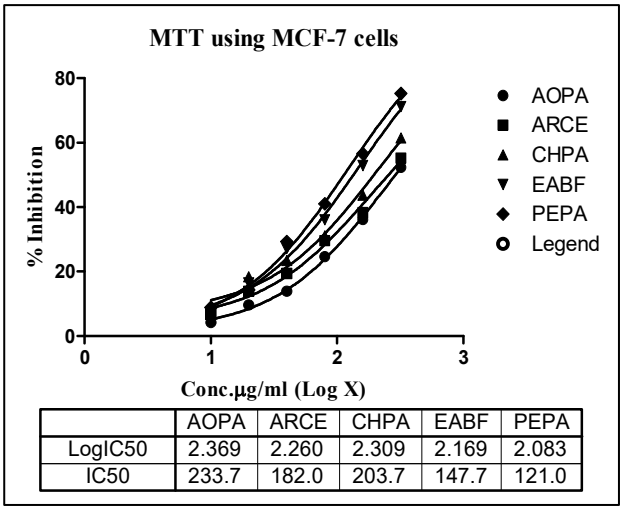
Table no: 2 Qualitative chemical analysis of various extracts of *Persea americana* Mill. leaves

Test	**Pet.Ether extract**	**Chloform extract**	**Ethylacetate extract**	**Aqueous extract**
Carbohydrates; Molisch test	-	-	-	+
Benedict's test	-	-	-	+
Fehling's test	-	-	-	+
Proteins; Millons test	-	-	-	+
Alkaloids; Mayer's test	-	+	-	-
Wagner's test	-	+	-	-
Hager's test	-	+	-	-
Dragendroff's test	-	+	-	
Steroids; Libermann Burchard test	+	+	-	-
Flavonoids; shinoda test	-	-	+	+
Tannins	-	-	-	+
Saponin glycoside	-	-	+	+

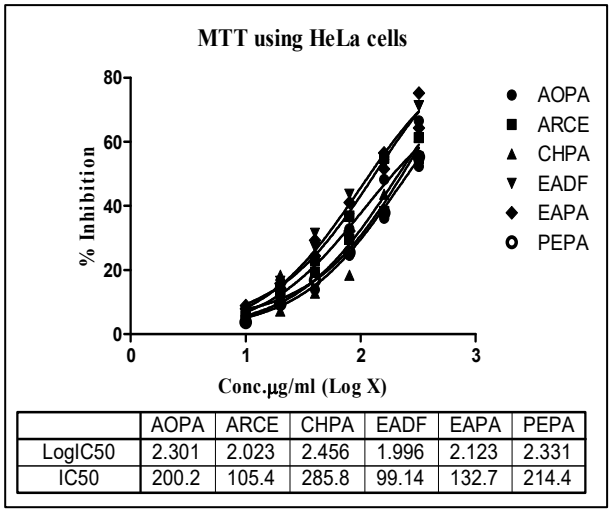
Table: 3 *In-vitro* anticancer activity by MTT Bioassay

Compound name	**MCF-7**				**HeLa**			
	Conc. µg/ml	**OD at 590nm**	**% Inhibition**	**IC_{50} µg/ml**	**Conc. µg/ml**	**OD at 590nm**	**% Inhibition**	**IC_{50} µg/ml**
Control	0	0.772	0		0	0.751	0	
AOPA	10	0.73927	4.24	**233.7**	10	0.71	5.46	**200.2**
	20	0.69766	9.63		20	0.661	11.93	
	40	0.66461	13.91		40	0.606	19.32	
	80	0.58193	24.62		80	0.504	32.84	
	160	0.49261	36.19		160	0.389	48.21	
	320	0.36778	52.36		320	0.251	66.58	
ARCE	10	0.72051	6.67	**182**	10	0.709	5.58	**105.4**
	20	0.66446	13.93		20	0.654	12.98	
	40	0.62208	19.42		40	0.578	23	
	80	0.5438	29.56		80	0.474	36.82	
	160	0.47648	38.28		160	0.339	54.9	
	320	0.34578	55.21		320	0.29	61.36	
CHPA	10	0.71572	7.29	**203.7**	10	0.719	4.23	**285.8**
	20	0.62995	18.4		20	0.696	7.26	
	40	0.59104	23.44		40	0.655	12.83	
	80	0.53253	31.02		80	0.613	18.36	
	160	0.43471	43.69		160	0.457	39.19	
	320	0.29707	61.52		320	0.34	54.79	
AQPA	10	0.71788	7.01	**147.7**	10	0.702	6.54	**99.14**
	20	0.64447	16.52		20	0.647	13.85	

	40	0.56263	27.12		40	0.514	31.53	
	80	0.49385	36.03		80	0.423	43.64	
	160	0.36369	52.89		160	0.347	53.75	
	320	0.2221	71.23		320	0.216	71.29	
EAPA	10	0.739	4.21	**IC_{50} was not calculated due to lesser % inhibition**	10	0.691	7.93	**132.7**
	20	0.705	8.72		20	0.632	15.83	
	40	0.671	13.08		40	0.568	24.42	
	80	0.646	16.38		80	0.509	32.18	
	160	0.583	24.48		160	0.364	51.55	
	320	0.534	30.83		320	0.268	64.37	
PEPA	10	0.70321	8.91	**121**	10	0.723	3.67	**214.4**
	20	0.66176	14.28		20	0.68	9.42	
	40	0.54565	29.32		40	0.624	16.85	
	80	0.45517	41.04		80	0.559	25.53	
	160	0.3352	56.58		160	0.467	37.81	
	320	0.1913	75.22		320	0.335	55.33	



	AOPA	ARCE	CHPA	EABF	PEPA
LogIC50	2.369	2.260	2.309	2.169	2.083
IC50	233.7	182.0	203.7	147.7	121.0



	AOPA	ARCE	CHPA	EADF	EAPA	PEPA
LogIC50	2.301	2.023	2.456	1.996	2.123	2.331
IC50	200.2	105.4	285.8	99.14	132.7	214.4

Figure 1: Significant findings of this study was that the *Persea americana* Miller shows that much stronger cytotoxic activity against MCF 7 cell line and HELA cell line using MTT bioassay.All the samples except EAPA has showed IC_{50} values of 233.7µg/ml, 182.0µg/ml, 203.7µg/ml, 147.7 µg/ml, and 121.0µg/ml respectively in MCF-7 cells. All the samples has showed IC_{50} value 200.2 µg/ml, 105.4µg/ml, 285.8 µg/ml, 132.7µg/ml and 214.4µg/ml respectively in HeLa . Good Ic50 values of PEPA and AQPA towards MCF7 and HELA cell line were repoted.

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