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Antioxidant and Cytotoxic Effects of *Morinda coreia* Buch-Ham Flower

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ABSTRACT

Aim and Objective: To evaluate the antioxidant and cytotoxic effects of *Morinda coreia* flower extracts.

Methods: The extracts of *Morinda coreia* flower was prepared by cold maceration. The antioxidant activity was screened by hydrogen peroxide, Phoshomolybdenum method and reducing power assay using ascorbic acid as a standard. The cytotoxicity effect on human breast adenocarcinoma cell line (MCF7) was evaluated by MTT assay.

Results: The antioxidant effect of extracts of *Morinda coreia* flower gets increased as concentration of extracts increases. The ethanolic extract of *Morinda coreia* possessed high antioxidant capacity and can be used as potent radical scavengers against deleterious damages caused by free radicals. MTT assay showed that *Morinda coreia* flower have cytotoxicity effect on human breast adenocarcinoma cell line (MCF7) and can be used as anticancer agents.

Conclusions: The results obtained indicates that extracts of *Morinda coreia* flower possessed potent antioxidant and cytotoxic activity.

Keywords: *Morinda coreia,* flower extracts, antioxidant, cytotoxic-MCF7 cell line.

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I.INTRODUCTION

The term antioxidant was used specifically to a chemical that prevents the consumption of oxygen. Natural antioxidants present in the plants scavenge harmful free radicals from our body. Antioxidants protects the cell against the damaging effects of reactive oxygen species (ROS) [1]. Oxidative stress has been implicated in the pathology of many diseases such as inflammatory conditions, diabetes, cardiovascular diseases, cancer, neurodegenerative diseases and aging [2,3]. The plants contain a wide variety of free radical scavenging molecules such as flavonoids, phenols, terpenoid, and vitamins. The plants have long history in the treatment of different cancer cells [4].

Many species of *Morinda* genus have been reported for various health disorders by Indian pharmacopoeia. *Morinda coreia* Buch-Ham is a evergreen shrub, flowering belongs to family Rubiaceae, native to southern Asia. The genus Morinda grows wild and is widely distributed in southern India. Many species of *Morinda* are available in India of which *Morinda coreia* predominantly grows as a weed in vacant agricultural land.

The leaves and fruits of *Morinda coreia* used as antimicrobial, anticonvulsant, antiulcer, antidiarrhoeial, anti-inflammatory, hepatoprotective, antioxidant[5-8]. From the scientific literature data, the folkloric uses of *Morinda coreia* flower in cancer studies are not available. So the present study was screened for antioxidant and cytotoxic effects of *Morinda coreia* flower extracts.

Our previous study indicates the pharmacognostical and phytochemical screening of *Morinda coreia* flower extracts. The present study is the continuation of the earliest work and is carried out to assess the antioxidant and cytotoxic effects of *Morinda coreia* flower extracts.

II.MATERIALS AND METHODS

2.1. Plant collection

Fresh plant material of *Morinda coreia* flower was collected from Virudhunagar district, Tamilnadu, India during September. The plant was identified and authenticated by Botanist Dr.Stephen, senior lecturer, American college of arts & science, Madurai.

2.2. Extraction

The collected fresh flower was shade dried, powdered and passed through sieve no 40. The coarse powder was stored in an air tight container. The extraction was carried out by cold maceration, in which 250 gm of coarse powder was defatted with 500 ml of petroleum ether for 4 hours. Then it was filtered and the residue was air dried. To the air dried residue, 500 ml of ethanol, was added and kept aside for 72 hours with intermittent shaking. After 72 hours the product was filtered and the filtrate was concentrated and dried at room temperature using rotovapour. The same process was carried out to the marc left after filtration to get ethyl acetate and aqueous extracts.

2.3. Determination of Antioxidant activities

2.3.1. By Hydrogen peroxide method.

To 1 mL of test solutions of different concentrations, 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 mL of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm

after 10 min. The reaction mixture without sample was used as blank. Sample blank was also prepared without reagents. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula

% inhibition = (A control – A sample) / A control X 100

The concentration of the sample required for 50 % reduction in absorbance (IC_{50}) was calculated using linear regression analysis [9].

2.3.2. By Phoshomolybdenum method.

An aliquot of 0.3 mL of different concentrations of samples was treated with 2.7 mL of the reagent (H2SO4, sodium phosphate and ammonium molybdate). In case of blank, 0.3 mL of methanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. The samples were cooled to room temperature; the absorbance of the aqueous solution of each concentration was measured at 695 nm against blank. The standard Ascorbic acid was treated in a similar manner. The antioxidant activity was expressed as the number of equivalents of Ascorbic acid (µg/g) [10].

2.3.3. By reducing power assay.

To 0.5 mL of plant extract of different concentrations solution (1 mg/mL) was mixed with 0.75 mL of phosphate buffer and 0.75 mL of 1 % potassium ferricyanide [$K_3Fe(CN_6)$] and incubated at 50°C for 20min. About 0.75 mL of 1 % Trichloro acetic acid was added to the mixture and allowed to stand for 10min. The whole mixture was then centrifuged at 3000 rpm for 10min. Finally 1.5 mL of the supernatant was removed and mixed with 1.5 mL of distilled water and 0.1mL of 0.1 % ferric chloride solution and the absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer was used as blank solution [11].

2.4. Determination of cytotoxic activity by MTT assay using MCF-7 cell line.

The human breast adenocarcinoma cell line (MCF7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% foetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a haemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml.

After 24 hours, the cells were treated with serial concentrations of the test samples. They were initially dissolved in Dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium.

Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and

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incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader [12.13].

The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100

The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample) / Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC_{50} was determined using Graph Pad Prism software.

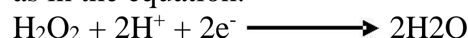
III.RESULTS

3.1. Hydrogen peroxide method

The principle is based on the capacity of the extract to decompose the hydrogen peroxide to water. H2O2 in the presence of O^{2-} can generate highly reactive hydroxyl radicals via the metal, the scavenging of H2O2 in cells is critical to avoid oxidative damage. Thus, the scavenging of hydrogen peroxide is an important antioxidant defense mechanism.

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^-$

The decomposition of hydrogen peroxide to water involves the transfer of electrons as in the equation.



$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H2O$

The results obtained for the scavenging activity against hydrogen peroxide are given in Table-1 and the graphical representation was presented in Fig-1.From the table, it can be seen that the EEMC (Ethanolic extract of *Morinda coreia*), EAEMC (Ethyl acetate extract of *Morinda coreia*), AEMC (Aqueous extract of *Morinda coreia*) showed the percentage inhibition 58.53±0.004, 62.54±0.01, 71.13±0.041µgm/ml, while ascorbic acid showed the percentage inhibition of 75.06±0.035 at a concentration of 50µgm/ml. The IC_{50} value for EEMC, EAEMC, AEMC & standard ascorbic acid was calculated using the linear regression analysis was found to be 44.72±0.002, 40.69±0.018, 35.69±0.043 and 32.55±0.031 µgm/ml.

Table 1-Percentage inhibition of standard ascorbic acid and *Morinda coreia* extracts by hydrogen peroxide method

Conc. in µgm/ml	Percentage inhibition			
	Ascorbic Acid	EEMC	EAEMC	AEMC
10	13.55±0.042	5.78±0.003	4.65±0.038	6.23±0.042
20	31.30±0.032	15.69±0.002	19.86±0.036	24.52±0.044
30	48.10±0.031	28.72±0.002	37.61±0.002	42.54±0.045
40	64.90±0.031	46.58±0.0021	49.96±0.022	58.53±0.042
50	75.06±0.035	58.53±0.004	62.54±0.012	71.13±0.041
IC_{50}	**32.55±0.031 µgm/ml**	**44.72±0.002 µgm/ml**	**40.69±0.018 µgm/ml**	**35.69±0.043 µgm/ml**

***mean of three readings** ± SEM

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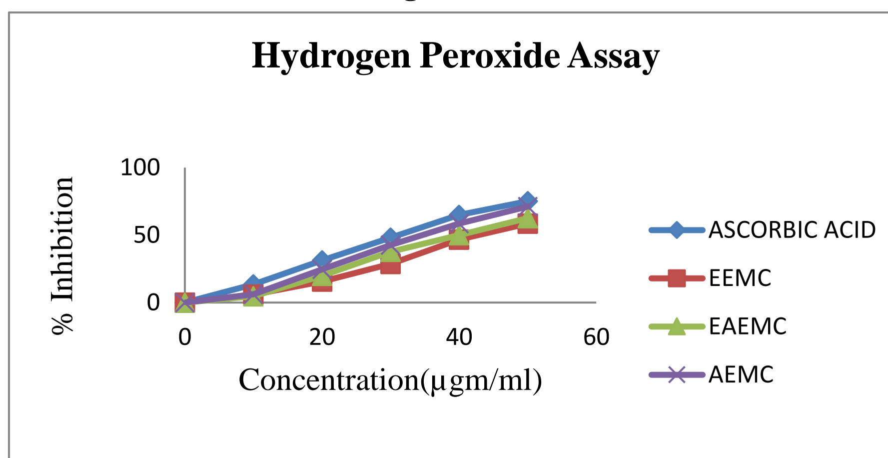
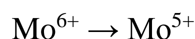
Fig-1



3.2. Phoshomolybdenum method

The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and by the subsequent formation of green phosphate Mo (V) complex at acidic pH which has a maximum absorption at 695 nm. This method is routinely used to determine total antioxidant activity of samples.



$$Mo^{6+} \rightarrow Mo^{5+}$$

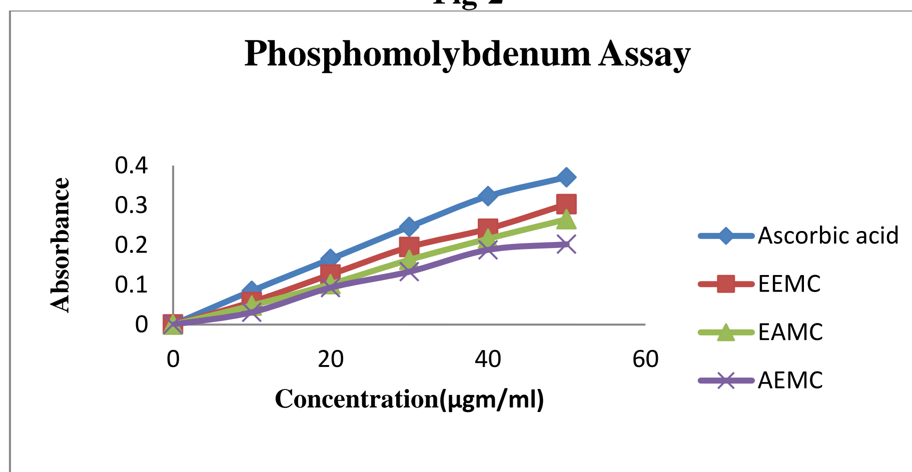
The results obtained for the different extracts of *Morinda coreia* flower by Phoshomolybdenum method were tabulated in Table-2 and the graphical representation was presented inFig-2. From the table, it can be seen that the EEMC, EAEMC, AEMC shows an absorbance of 0.303±0.005, 0.265±0.018, 0.202±0.021 at a concentration of 50 µgm/ml respectively, while the standard ascorbic acid showed an absorbance of 0.371±0.005 at a concentration of 50 µgm/ml.

Table-2 Absorbance of different extracts of *Morinda coreia* and standard ascorbic acid by Phoshomolybdenum method

Conc.in µgm/ml	Absorbance			
	Ascorbic acid	**EEMC**	**EAMC**	**AEMC**
10	0.085±0.005	0.057±0.003	0.048±0.004	0.031±0.016
20	0.165±0.004	0.126±0.005	0.102±0.002	0.093±0.011
30	0.246±0.008	0.195±0.006	0.163±0.005	0.133±0.006
40	0.323±0.004	0.241±0.008	0.216±0.002	0.188±0.008
50	0.371±0.005	0.303±0.005	0.265±0.018	0.202±0.021

***mean of three readings** ± SEM

Fig-2



3.3. Reducing power assay

The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent formation of Prussian blue colour with ferric chloride. The absorbance of the blue complex is measured at 700 nm.

The results obtained for the different extracts of *Morinda coreia* flower extracts and standard ascorbic acid by reducing power assay are tabulated in Table-3 and the graphical representation was presented in Fig-3. From the table, it can be seen that the EEMC, EAEMC, AEMC showed an absorbance of 0.383±0.007, 0.277±0.003, 0.226±0.01 at a concentration of 50 µgm/ml respectively, while the standard ascorbic acid showed an absorbance of 0.552±0.007 at a concentration of 50 µgm/ml. The extract showed a dose dependent reducing ability.

Table-3 Reducing Power Assay of *Morinda coreia* extracts and Standard Ascorbic Acid

S.NO	Conc. in µgm/ml	Absorbance			
		Ascorbic acid	EEMC	EAMC	AEMC
1	10	0.098±0.012	0.064±0.002	0.059±0.002	0.032±0.014
2	20	0.203±0.003	0.135±0.004	0.104±0.005	0.083±0.021
3	30	0.317±0.002	0.198±0.002	0.157±0.001	0.132±0.012
4	40	0.431±0.009	0.267±0.003	0.209±0.004	0.164±0.016
5	50	0.552±0.007	0.383±0.007	0.277±0.003	0.226±0.011

***mean of three readings** ± SEM

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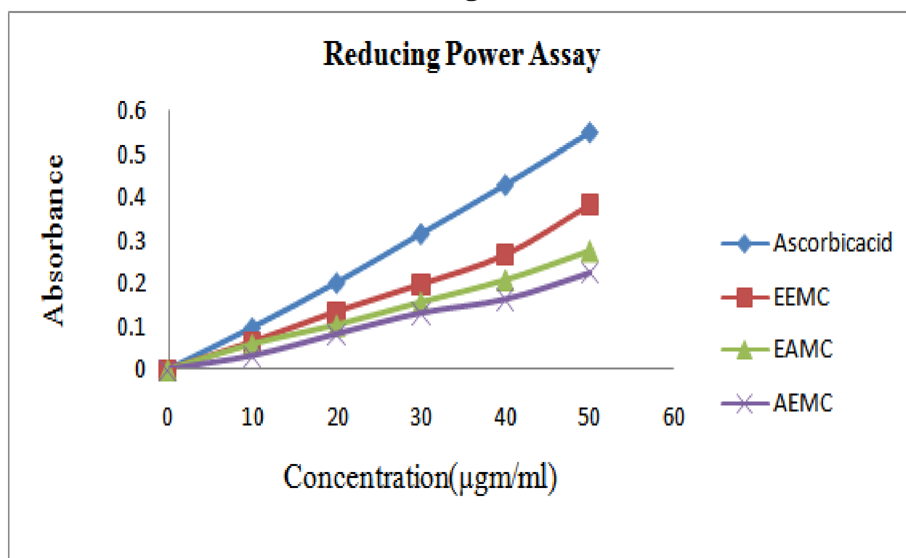
Fig-3



3.4. Cytotoxicity studies.

Cytotoxicity has been assessed using different concentrations (12.5, 25, 50 100 and 200µg/ml) of the extracts (EEMC, EAEMC and AEMC) by MTT assay. The cytotoxic effect of extracts were observed and compared with control group. The *Morinda coreia* flower extracts showed slight decrease in cell viable count compared to control group at a increasing dose dependent manner. The percentage of cell inhibition was higher in EAEMC (73.83) when compared with EEMC (18.55) and AEMC (16.46) at 200µg/ml concentrations. The cytotoxic effects of the *Morinda coreia* extracts were depicted in Table-4and Fig.4,5.

Table-4 Cytotoxicity of *Morinda coreia* flower extracts against MCF7 cancer cell lines.

Conc (µgm/mL)	Absorbance			% of Cell viability			% of Cell Inhibition		
	EEMC	EAEMC	AEMC	EEMC	EAEMC	AEMC	EEMC	EAEMC	AEMC
12.5	0.354	0.352	0.356	101.142	100.571	101.714	-1.142	-0.571	-1.714
25	0.343	0.338	0.354	98	96.571	101.142	2	3.429	-1.142
50	0.338	0.314	0.349	96.571	89.714	99.714	3.429	10.286	0.286
100	0.299	0.242	0.340	85.428	69.142	97.142	14.572	30.858	2.858
200	0.285	0.091	0.292	81.428	26	83.428	18.572	74	16.572
Control	0.350								

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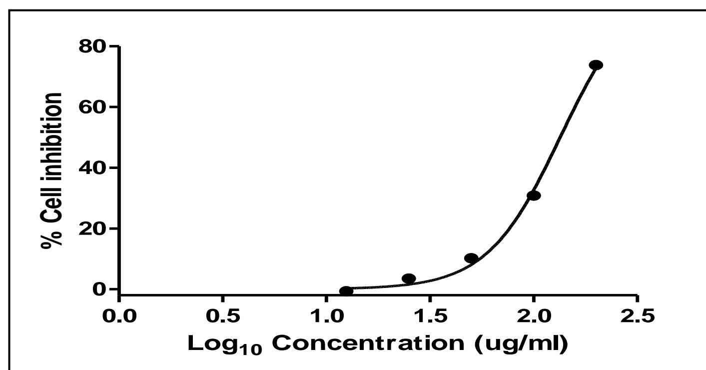
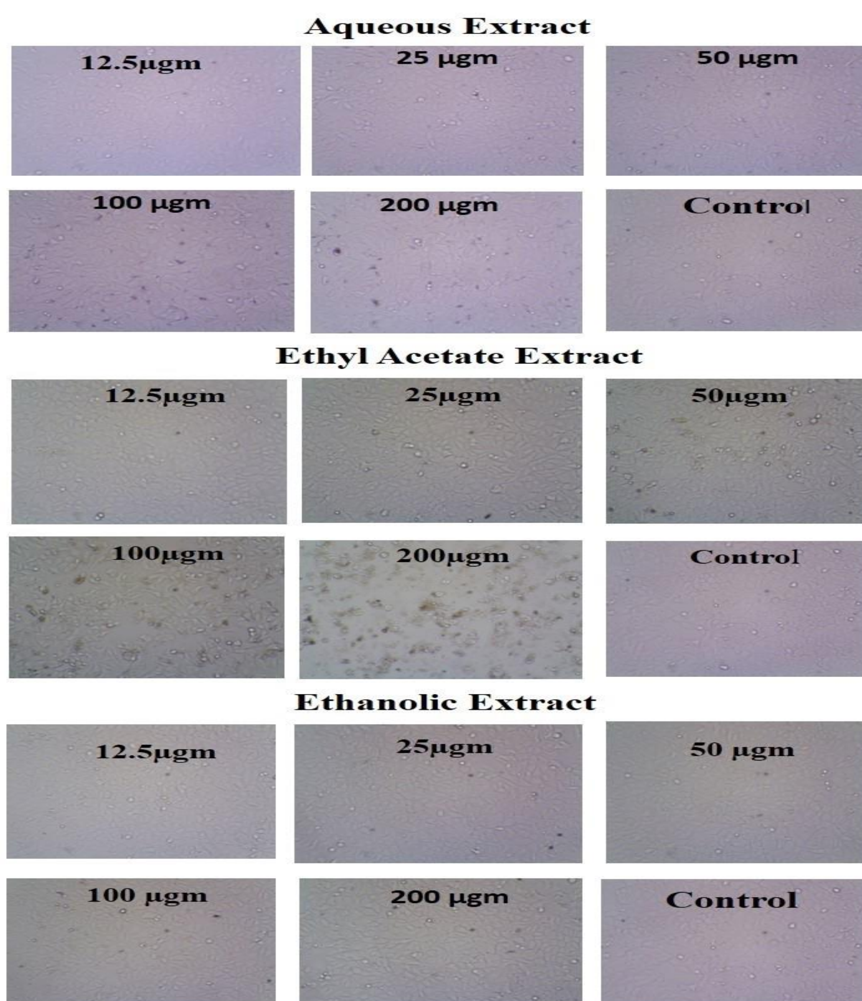
Fig-4



IC $_{50}$=134.2 µg/ ml R^2=0.9964

Fig-5.Cytotoxicity for extracts of *Morinda coreia* flower



CONCLUSION

The invitro antioxidant activity for various extracts (EEMC, EAEMC and AEMC) of *Morinda coreia* flower was evaluated, by hydrogen peroxide, reducing power assay and Phoshomolybdenum method. The EEMC showed potent antioxidant activity than EAMC and AEMC.

The Cytotoxicity has been assessed using different concentrations of the extracts (EEMC, EAEMC, and AEMC) by MTT assay. The results predicted that EAEMC, flower was more active against MCF7 cell line which possessed good anticancer effects. Further experimental analysis on these plants would definitely reveal the important chemical constituents responsible for activity.

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