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IN VITRO ANTICANCER ACTIVITY OF ETHYL ACETATE EXTRACT AND GREEN NANOPARTICLE SYNTHESIZED FROM *CURCULIGO ORCHIOIDES* GAERTN - AN ENDANGERED MEDICINAL PLANT

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Keywords:

Curculigo orchioides, Ethyl acetate extract, Nanoparticle, MTT assay, MCF-7 cell line

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ABSTRACT: The present study aimed at to study the antioxidant and anticancer activity of ethyl acetate extract and nanoparticles synthesized from *Curculigo orchioides*. The ethyl acetate extract andnanoparticle synthesized from *C. orchioides* were used in various antioxidant assays such as DPPH, hydroxyl radicals, hydrogen peroxide and nitric oxide radical scavenging activities. The synthesis of silver nanoparticles was characterized by UV-Vis spectro-photometer, X-ray diffractometer (XRD) and Scanning Electron Microscope (SEM). The maximum growth inhibitory effects (66.12% and 71.28%) on MCRF-7 cell line were observed in ethyl acetate extract and nanoparticle at 80µg/ml concentration after 48 hr treatment.

INTRODUCTION: Cancer is a dreadful disease and treating this disease is of great importance to human health care. Though many therapeutic methods are available including chemotherapy to treat cancer, there is a high systemic toxicity and drug resistance limit the success of the treatment [1]. Recently chemotherapeutic agents are used with the combination of phytochemical agents. This would enhance the efficacy while reducing toxicity to normal tissues. Free radicals react with purines, pyrimidine, and chromatin protein leading to base modifications, unstable genomes and genetic alterations. These transformed cells have altered levels of cell cycle and apoptosis signalling molecules thereby resulting in uncontrolled cell proliferation and tumour formation [2].

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Now a day, nanoparticle synthesis and the study of their size and properties are fundamental importance in the advancement of recent research. It was found that the catalytic properties of metal nanoparticles depend on their size, shape and chemical surroundings [3]. Silver nanoparticle has gained much interest in the field of medicine because of their chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal, anticancer and anti-inflammatory activities.

In the present study nanoparticles has been synthesized using *Curculigo orchioides* rhizome extract and screened for anticancer activity. *Curculigo orchioides* is an endangered medicinal plant found in the tropics of Asia. It is a perennial herb with tuberous root and lateral root which are blackish brown externally and cream internally. It contains substances like mucilage, phenolic glycosides, saponins and aliphatic compounds. The present study was aimed at to evaluate the effect of ethyl acetate extract and nanoparticle of *C.*

orchioides on antioxidant potential as well as MCF-7, a breat cancer cell line.

MATERIALS AND METHODS:

Extraction: The powdered material of rhizome (30g) was extracted with ethyl acetate. The extract was dried under reduced pressure by using rotary evaporator.

Nanoparticle Synthesis: Silver nitrate is used in nanoparticle synthesis of *Curculigo orchioides*. 1.5 g of the *C. orchioides* rhizome powder was boiled in 100ml of de-ionized water. 2.5ml of ammonium solution was added to 5ml of 1mM $AgNO_3$ solution, followed by addition of plant extract 1-10 ml and the final volume was adjusted to 50ml by adding the appropriate amount of de-ionized water. For silver nanoparticles, the solution turned from yellowish to bright yellow and to dark brown.

Silver Nanoparticle (AgNP) Synthesis: The solution (1mM) of silver nitrate ($AgNO_3$) was prepared in 250mL Erlenmeyar flasks and rhizome extract was added for reduction into Ag^+ ions. The composite mixture was kept exposed to sunlight for 30min. The synthesis of AgNPs was furthermore confirmed by spectrophotometric analysis.

Charaterization of AgNPs:

UV-Vis Spectra Analysis: Test samples (1mL) of the suspension were collected periodically to monitor the completion of bio reduction of Ag^+ in aqueous solution. The solutions were scanned in UV- visible (Vis) spectra, between wavelengths of 200 to 700 nm in a spectrophotometer (Hitachi), having a resolution of 1nm.

X-ray Diffraction (XRD): XRD patterns were recorded using powder X-ray diffractometer (model Rigakuminiflex II) to analyze phase formation and purity.

Scanning Electron Microscope (SEM) Analysis: The synthesized AgNPs were centrifuged at 4,000 rpm for 15min and the supernatants were discarded. The pellet was mixed properly and carefully placed on a glass cover slip followed by air-drying. The samples were then gold coated and the images of NPs were obtained in a scanning electron microscope (JEOL, Akishima-shi, Japan - model No.JFC-1600).

DPPH Radical Scavenging Assay: DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging activity of ethyl acetate extract and nanoparticles synthesized from *Curculigo orchioides* were determined with the method proposed by Sanchez *et al*., (1998). The ethyl acetate extract, nanoparticle and standard reference compounds were dissolved with 99% ethanol at various concentrations. 1 ml of various concentrations (50-1000µg/ml) of the ethyl acetate extract, nanoparticle and standard reference compounds were mixed with 1ml of 0.2 mM DPPH separately and made up using 99% ethanol to a final volume of 3ml. The mixture was incubated for 30 min at room temperature. The absorbance was recorded at 517nm.Scavenging activity was calculated as following:

Radical scavenging (%): (OD control-OD test sample)/OD control x100

Hydroxyl Radical Scavenging Assay: The Hydroxyl radical scavenging activity of the ethyl acetate extract and nanoparticle synthesized from *C. orchioides* were measured according to the modified method of Chung *et al*., (1997). The Fenton reaction mixture containing 200µl of 10mM $FeSO_4.7H_2O$, 200 µl of 10mM EDTA and 200µl of 10mM 2-deoxyribose was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) containing 200µl of ethyl acetate extract and nanoparticle of *C. orchioides*. Then, 200µl 10mM H_2O_2 was added to the mixture and incubated for 4h at 37 °C. After incubation, 1ml of 1% TBA were added and placed in a boiling waters bath for 10 smin. The resultant mixture was then cooled and centrifuged at 8000rpm. The absorbance was measured at 532nm.

Radical scavenging (%): (OD control-OD test sample)/OD control x100

Hydrogen peroxide Radical Scavenging Assay: The Hydrogen radical scavenging activity of the ethyl acetate extract and nanoparticle synthesized from *C. orchioides* were measured according to the method of Ruch *et al*., (1989). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). The ethyl acetate extract and nanoparticle (50-1000µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6ml, 40mM).

The absorbance of hydrogen peroxide at 230nm was measured after 10min against a blank solution containing phosphate buffer without hydrogen peroxide using a UV-VIS spectrophotometer.

Radical scavenging (%): (OD control-OD test sample)/OD control x100

Nitric - oxide Radical Scavenging Assay: Nitric oxide from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was determined by the Griess reaction. The reaction mixture (3ml) containing 10mM sodium nitroprusside in phosphate-buffered saline, ethyl acetate extract and nanoparticle and the reference compounds at different concentrations (50, 100, 200, 400, 500 and 1000µg/ml) were incubated at 25 °C for 15min. A 0.5ml Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediaminedi - hydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was determined at 546nm. The inhibition percentage of the nitric oxide was measured by the following formula:

Radical scavenging (%): (OD control-OD test sample)/OD control x100

Cell Line: The human breast adeno-carcinoma cell line (MCF7) was obtained from National Centre for Cell Science (NCCS), Pune, India and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37 °C, 5% CO_2, 95% air and 100% relative humidity. Maintenance of cultures was passaged weekly, and the culture medium was changed twice a week.

Cell Treatment Procedure: The monolayer cells were detached with trypsin-ethylenediamine-tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 °C, 5% CO_2, 95% air and 100% relative humidity. After 24hr the cells were treated with serial concentrations of the test samples. They were initially dissolved in dimethyl-sulfoxide and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100µl of these different sample dilutions were added to the appropriate wells already containing 100µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48hr at 37 °C, 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.

MTT Assay: 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.After 48hr of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4hr. 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. The percentage cell growth was then calculated with respect to control as follows:

$$\% \text{ Cell growth} = [A]_{Test} / [A]_{control} \times 100$$

The % cell inhibition was determined using the following formula:

$$\% \text{ Cell Inhibition} = 100 - Abs_{(sample)} / Abs_{(control)} \times 100$$

Annexin-V FITC Assay: MCRF-7 cell line was treated with 10 and 20µg/ml of ethyl acetate extract and nanoparticles separately for 24hr. At the end of the treatment, cells were washed with phosphate buffered saline (PBS) and suspended in binding buffer (10mM HEPES/NaOH pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). Aliquots of cells (100µl) were mixed with 5µl of Annexin FITC (fluoresce-inisothiocyanate-conjugate) and incubated for 15 minutes at room temperature in dark and stained with propedium iodide (5µg/ml). The cells were then gently vortexed and analyzed using Becton

Dickinson FACS caliber. Early apoptotic cells were defined as those cells exhibiting a fluorescein isothiocynanate-conjugate and incubated for 15 minutes at room temperature in dark and stained with propidium iodide (5µg/ml). The cells were then gently vortexed and analyzed using Becton Dickinson FACS caliber. Early apoptotic cells were defined as those cells exhibiting a fluoreseiniso-thiocyanate-conjugated annexin V-positive and propodium iodide-negative staining pattern; necrotic cells exhibit propidium iodide positive and annexin-V FITC negative.

RESULTS AND DISCUSSION:

AgNP Characterization:

UV-Vis Analysis: Silver nanoparticles (Ag NPs) appear yellowish brown in colour in aqueous silver nitrate solution. The UV-Vis spectra recorded after time intervals of 15min, 30min, 45min, 60min and 24hr. Absorption spectra of Ag NPs formed in the reaction media has maximum absorption in the range of 425 to 475nm. UV-Vis spectra confirmed that formation of Ag NPs occurred within 30 min.

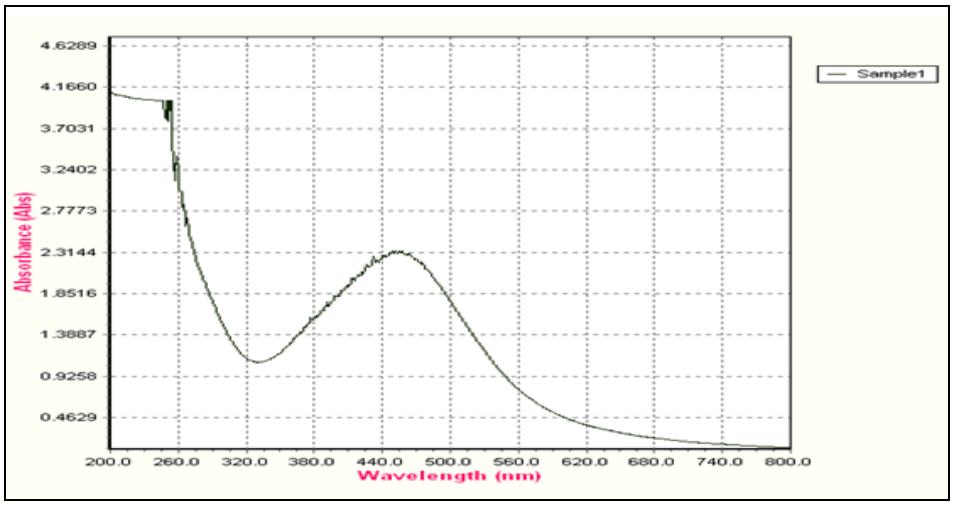


FIG. 1: UV-VIS SPECTRA OF SILVER NANOPARTICLES SYNTHESIZED USING *CURCULIGO ORCHIOIDES* XRD

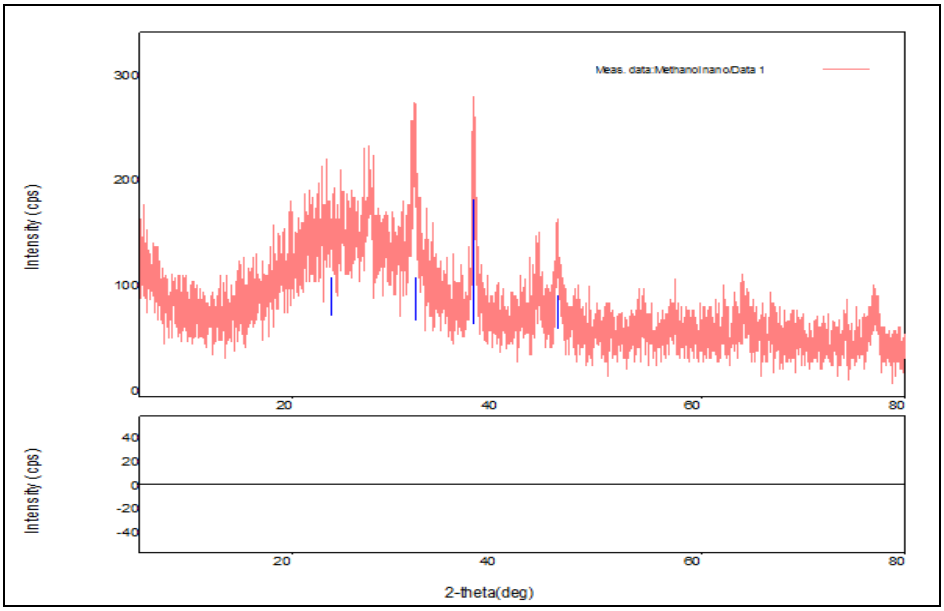


FIG. 2: X-RAY DIFFRACTION PATTERN OF THE SILVER NANOPARTICLES WERE SYNTHESIZED FROM *CURCULIGO ORCHIOIDES*

Scanning Electron Microscope (SEM) Analysis: The rhizome extract of *Curculigo orchioides* formed cuboidal AgNPs. The SEM image of the AgNP is shown in **Fig. 4**. This could be due to availability of different quantity and nature of capping agents present in the rhizome extract.

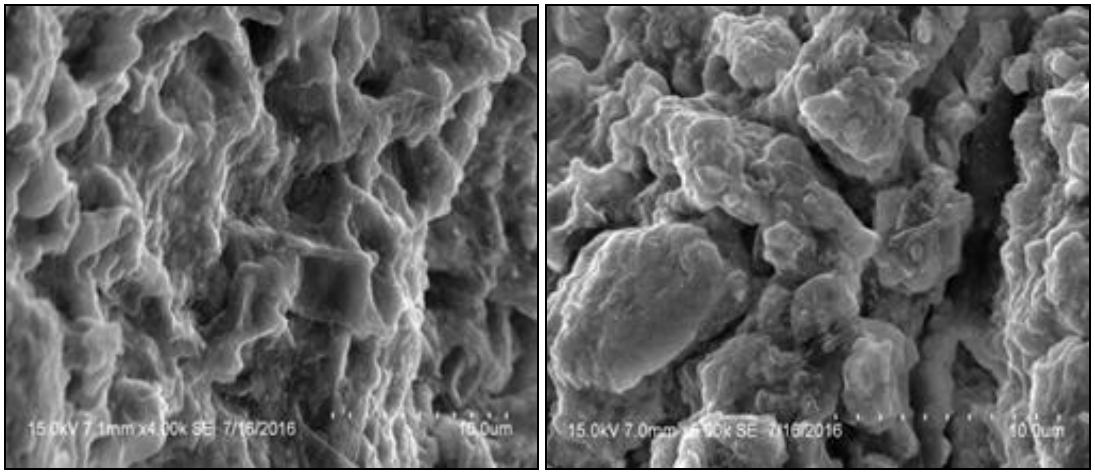


FIG. 3: SEM IMAGES OF THE SILVER NANOPARTICLES SYNTHESIZED FROM *CURCULIGO ORCHIOIDES*

Antioxidant Property: The free radical scavenging activity of ethyl acetate extract and nanoparticle were studied by using DPPH, Hydroxyl, Hydrogen peroxide and Nitric oxide respectively. The DPPH radical scavenging activity is a standard procedure for the evaluation of antiradical activity.

In DPPH free radical scavenging activity, the nanoparticle revealed to high percentage of inhibition (62.11 ± 0.32%) at 1000µg/ml, whereas the ethyl acetate extracts showed 61.28 ± 0.26% (**Table 1**). In hydroxyl free radical scavenging activity, nanoparticle showed high activity 64.11 ± 0.32% at 1000µg/ml whereas it showed 59.19 ± 0.38% in ethyl acetate extract (**Table 2**). In the case of hydrogen peroxide free radical scavenging activity, the nanoparticle and ethyl acetate extract showed inhibition percentage of 61.29 ± 0.61% and 52.58 ± 0.26% at 1000µg/ml (**Table 3**). For nitric oxide scavenging activity, nanoparticle showed high activity 62.11 ± 0.32% whereas ethyl acetate extracts showed least activity 61.28 ± 0.26% (**Table 4**).

Hydroxyl radicals can accelerate the peroxidation of the polyunsaturated fatty acids in the cell membrane, generating malonaldehyde which is easy to react with phospholipid and hence the characteristics of the cell membrane can be changed. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contribute to aging, carcinogenesis, mutagenesis and cytotoxicity. The ability of the extract to quench hydroxyl radicals seems to directly relate to the prevention of propagation of the process of lipid peroxidation, and the extract seems to directly relate to the prevention of propagating of the process of lipid peroxidation, and the extract seems to be a good scavenger of active radical species, thus reducing the rate of chain reaction.

Hydrogen peroxide can sometimes be toxic to cell because it may give rise to OH radical in the cells. Addition of hydrogen peroxide to cells in culture can lead to transition metal ion dependent OH radicals mediated DNA damage.

Nitric oxide is a free radical, and plays many roles as an effect or molecule in diverse biological systems including neuronal signalling and regulation of cell mediated toxicity. Nitric oxide has many beneficial functions in organisms, including regulation of vascular tone, ventilation, hormone secretion, inflammation, immunity and neurotransmission, cytotoxic or cytostatic to host cells, and to act as a toxic radical. In the present study, nitric oxide was scavenged more by nanoparticle synthesized. Thus ethyl acetate extract and nanoparticle synthesized were found to inhibit free radical scavenging activity such as DPPH, Hydrogen peroxide and Nitric oxide.

TABLE 1: DPPH RADICAL SCAVENGING ASSAY

Conc. µg/ml	% inhibition of DPPH free radical		
	Ethyl acetate	nanoparticle	ascorbic acid
50	14.27±0.38	20.16±0.22	73.29±0.16
100	16.85±0.48	28.11±0.11	76.54±0.37
200	27.37±0.68	45.08±0.68	84.26±0.19
400	48.28±0.84	58.61±0.22	91.76±0.72
500	56.48±0.51	63.23±0.31	93.68±0.47
1000	61.28±0.26*	62.11±0.32	95.39±0.46

Values are mean of three different experiment ±SD
*values indicate that the values are siginificant

TABLE 2: HYDROXYL RADICAL SCAVENGING ACTIVITY

Conc. µg/ml	% inhibition of hydroxyl radical scavenging activity		
	Ethyl acetate	nanoparticle	ascorbic acid
50	9.27±0.11	5.53±0.13	87.63±0.38
100	11.28±0.26	18.47±0.26	88.57±0.52
200	41.26±0.36	44.08±0.38	88.27±0.43
400	50.26±0.65	57.61±0.21	92.17±0.43
500	56.87±0.55	62.22±0.31	95.63±0.37
1000	59.19±0.38*	64.11±0.32	97.69±0.31

Values are mean of three different experiment ±SD
*values indicate that the values are siginificant

TABLE 3: HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY

Conc. µg/ml	%inhibition of hydrogen peroxide radical scavenging activity		
	Ethyl acetate	nanoparticle	ascorbic acid
50	2.76±0.29	9.52±0.61	46.12±0.25
100	11.28±0.37	18.19±0.51	55.59±0.48
200	26.59±0.65	32.71±0.61	69.36±0.72
400	41.47±0.39	46.68±0.49	71.48±0.59
500	48.29±0.75	52.81±0.67	73.49±0.39
1000	52.58±0.26*	61.29±0.61	83.69±0.29

Values are mean of three different experiment ±SD
*values indicate that the values are siginificant

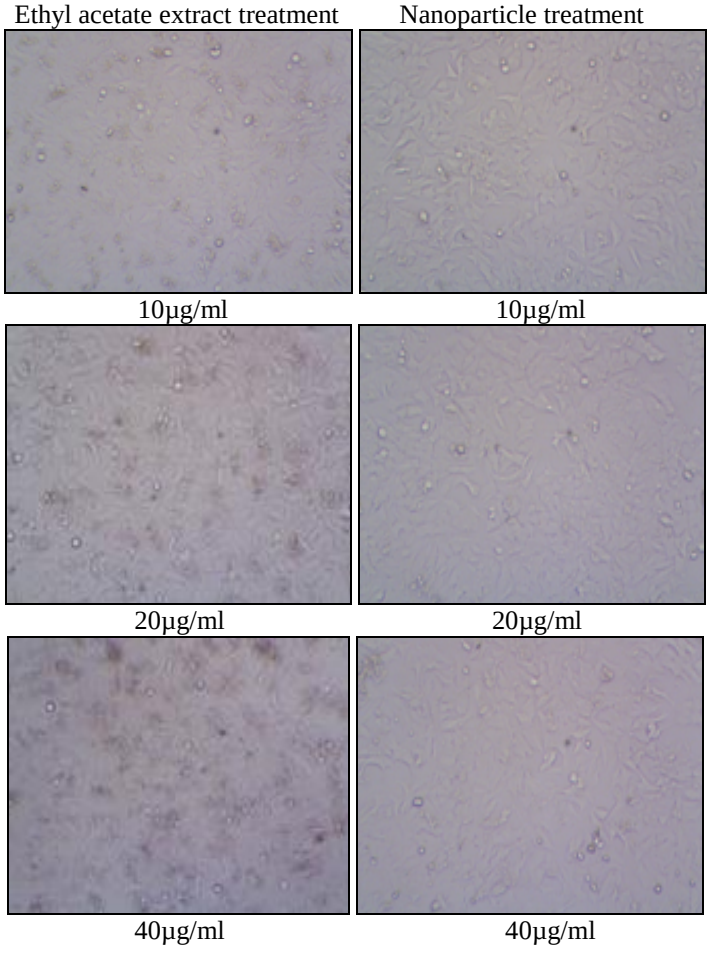
TABLE 4: NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

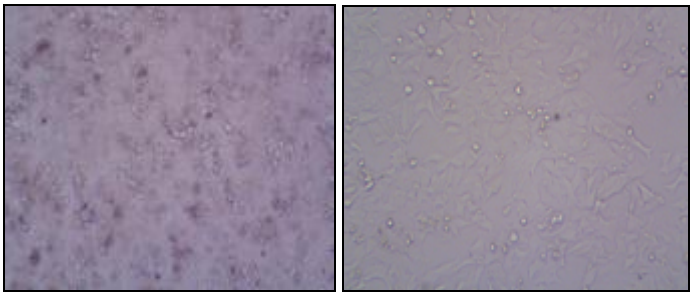
Conc. µg/ml	% inhibition of nitric oxide radical scavenging activity		
	Ethyl acetate	nanoparticle	ascorbic acid
50	14.27±0.38	20.16±0.22	73.29±0.16
100	16.85±0.48	28.11±0.11	76.54±0.37
200	27.37±0.68	45.08±0.68	84.26±0.19
400	48.28±0.84	58.61±0.22	91.76±0.72
500	56.48±0.51	63.23±0.31	93.68±0.47
1000	61.28±0.26*	62.11±0.32	95.39±0.46

Values are mean of three different experiment ±SD
*values indicate that the values are siginificant

Cytotoxic Activity: Cancer specific cytotoxic effects of the ethyl acetate extract and nanoparticle from *Curculigo orchioides* were studied using human breast adenocarcinoma cell line (MCF7). The degree of toxicity of these test compounds towards MCF7 cell line was determined using MTT assay. The colorimetric assay is based on the ability of the viable cells to reduce a soluble yellow tetrazolium salt (MTT) to blue formazan crystals.

MCF7 CELL LINE





80µg/ml 80µg/ml

FIG. 4: CYTOTOXIC EFFECT OF ETHYL ACETATE EXTRACT AND NANOPARTICLE FROM *CURCULIGO ORCHIOIDES* AGAINST MCRF7 CELL LINE

The IC_{50} values of cytotoxic activities were calculated from the dose survival curves obtained after period of compounds treatment. Ethyl acetate extract and nanoparticle from *Curculigo orchioides* exhibited cytotoxic effect towards MCRF-7 cell line in a dose and time dependant manner compared with untreated control cell line (**Fig. 1**). The viability of MCRF7 was significantly decreased at 80µg/ml concentration. Maximum growth inhibitory effects (66.12% and 71.28%) on MCRF7 cell line were observed in ethyl acetate extract and nanoparticle from *Curculigo orchioides* at 80µg/ml concentration after 48hr treatment.

The ethyl acetate extract and nanoparticle from *Curculigo orchioides* were tested for cytotoxicity using MCRF7 cell line. Ethyl acetate extract and nanoparticle from *Curculigo orchioides* were found to be cytotoxic for the cell line in a concentration and time dependent manner.

Ethyl acetate extract from *Curculigo orchioides* showed 21.67, 40.61, 52.62 and 66.12% cyto-toxicity at 80 µg/ml concentrations for 6, 12, 24 and 48hr incubation against MCRF7 cells (**Table 5** and **Fig. 1**). The nanoparticle from *Curculigo orchioides* was found that there were 20.17, 42.33, 62.14 and 71.66% cytotoxicity towards MCRF7 cells at 80µg/ml concentration for 6, 12, 24 and 48 hr incubation respectively.

The IC_{50} values of ethyl acetate extract and nanoparticle against MCRF7 were not detectable up to the tested concentrations for 12 hours incubation periods (**Table 3**).

Annexine V FITC Assay: Apoptosis is a normal physiologic process for removal of unwanted cells. Apoptosis includes translocation of membrane phosphatidylserine (PS) from the inner side of the plasma membrane to the surface. Annexin V, a Ca^{2+} - dependent phospholipid-binding protein, has high affinity for PS, and fluoro-chrome-labelled Annexin V can be used for the detection of exposed PS.

Annexin V FITC assay can also be used as a starting point for creating custom experiments and assays to suit the needs of researchers. These user-defined assays can then be run in a work list, or deployed to other BD FACS Verse cytometers within the laboratory or to an external site [4].

It was found that the cytotoxic activity of nanoparticle from *C. orchioides* is higher than the ethyl acetate extract. It showed the highest activity with IC_{50} value at 38.05µg/ml and 16.06µg/ml in 48 hr incubation period against MCRF7 cell lines of ethyl acetate extract and nanoparticle respectively. The ability of the test compounds to induce apoptosis was studied by staining the treated cells with annexine VFITC and propidium iodide. MCRF7 cells were placed in a 25 cm^2 flask at a density of $1x10^7$ cells and allowed to form confluence and the cells were tested with 10 and 20 µg/ml concentrations in 1% serum containing medium. Control cells were also treated with the same as the treatment group without the compound and the final concentration of DMSO was kept below 1%.

The ethyl acetate extract from *C. orchioides* was able to induce apoptosis in MCRF 7 cells with 2.11% of the cells at early apoptosis and 1.22% of cells at late apoptosis. Nanoparticle from *C. orchioides* was able to induce apoptosis in MCRF7 with 2.62% of the cells at early apoptosis and 1.68% of cells at late apoptosis for MCRF7 (**Table 4** and **5**).

TABLE 5: CYTOTOXIC EFFECT OF ETHYL ACETATE EXTRACT FROM *CURCULIGO ORCHIOIDES* AGAINST MCF7

Fraction µg/ml	MCF7 10,000 cells / well			
	6h	12h	24h	48h
10	02.02 ± 1.02	06.66 ± 1.20	26.44 ± 1.21	30.12 ± 1.11
20	04.66 ± 1.11	13.66 ± 1.24	34.23 ± 1.66	41.12 ± 1.23
40	16.32 ± 0.68	18.66 ± 1.11	40.18 ± 0.22	50.66 ± 0.66
80	21.67 ± 1.88	40.61 ± 1.66	52.62 ± 1.83	66.12 ± 0.21

Values are mean of three different experiment ± SD

TABLE 6: CYTOTOXIC EFFECT OF NANOPARTICLE FROM *CURCULIGO ORCHIOIDES* AGAINST MCF7

Fraction µg/ml	MCF7 10,000 cells / well			
	6h	12h	24h	48h
10	02.11 ± 1.11	17.44 ± 1.71	30.21 ±0.30	41.11 ±0.80
20	06.18 ± 1.02	29.62 ± 1.12	41.52 ±0.12	52.12 ±1.22
40	11.65 ±0.14	32.12 ±1.02	51.12 ±0.81	63.23 ±0.11
80	20.17 ±0.81	42.33 ±1.26	62.14 ±1.02	71.66 ±0.12

Values are mean of three different experiment ± SD

TABLE 7: IC_{50} CONCENTRATIONS OF THE ETHYL ACETATE EXTRACT AND NANOPARTICLE FROM *CURCULIGO ORCHIOIDES*

Sample	IC_{50} values in µg/ml		
	12h	24h	48h
Methanol extract	>80	50.60	38.05
Nanoparticle	>80	40.51	16.06

Values are mean of three different experiment ± SD

TABLE 8: PERCENTAGE OF APOPTOSIS INDUCED BY ETHYL ACETATE EXTRACT FROM *CURCULIGO ORCHIOIDES*

Concentration	Viable cells	Necrotic cells	Late apoptotic	Early apoptotic
Control	100	-	-	-
10µg/ml	82.66	10.11	1.22	2.11
20µg/ml	71.56	14.12	4.21	3.22

Values are mean of three different experiment ± SD

TABLE 9: PERCENTAGE OF APOPTOSIS INDUCED BY NANOPARTICLE FROM *CURCULIGO ORCHIOIDES*

Concentration	Viable cells	Necrotic cells	Late apoptotic	Early apoptotic
Control	100	-	-	-
10µg/ml	83.21	09.21	1.68	2.62
20µg/ml	72.62	13.67	4.22	4.62

Values are mean of three different experiment ± SD

Antioxidants have a well-documented role in numerous ailments including diabetes, cancer and despite the presence of endogenous defense mechanisms, an imbalance in the redox system leads to oxidative stress and damage the cellular molecules leading to carcinogenesis [5, 6]. Natural plants are a cheap source for the extraction of antioxidant compounds, thus providing important economic advantage. In the present study cytotoxicity was performed to determine the toxicity of ethyl acetate extract and nanoparticle from *Curculigo orchioides*. It was found that ethyl acetate extract and nanoparticle from *Curculigo orchioides* showed cytotoxicity towards MCRF7 cell lines with IC_{50} of 38.05µg/ml and 16.06µg/ml. recently traditional medicine (Phyto-therapy) is commonly used to treat various diseases besides modern medicine. People prefer to use herbal products because synthetic drugs can cause different side, effects, so that about 80% of the world population uses medicinal plants [7].

The use of bioassay offers various scientific strategies like screening of test compounds obtained from plants, which are often used in phytochemical research. In the *in vitro* evaluation method for natural products the biological activity has changed in the past few years, one of the recent developments is comet assay, which gives a ratio between the viable cells in cell culture to total cells in the culture [8]. These techniques are considered rapid and economical for the evaluation of anticancer activity of compounds [9].

Recently, a plant derived bioactive substance that is capable of selectively arresting cell growth in tumour cells has received considerable attention in cancer chemopreventive approaches [10, 11]. Many chemical substances derived from medicinal plants are known to be effective and versatile chemo-preventive agents in a number of experimental models of carcinogenesis.

CONCLUSION: In the present study ethyl acetate extract and nanoparticle synthesized from *C. orchioides* possess significant antioxidant and

anticancer activities. The antioxidant ability could be attributed to the phenolic compounds. From the analysis reported from above it can be concluded that ethyl acetate extract and nanoparticle synthesized from *Curculigo orchioides* possess anticancer properties against breast cancer cell line (MCF-7). Nanoparticle synthesized from *Curculigo orchioides* showed higher anticancer activities than ethyl acetate extract. Further experimental analysis on these plants would definitely reveal the important chemical constituents responsible for treating the cancer.

REFERENCES:

1. Gali K, Ramakrishnan G, Kothai R and Jaykar B: *In-vitro* Anti-Cancer activity of Methanolic extract of leaves of *Argemone mexicana* Linn. International Journal of Pharm Tech Research 2011; 3(3): 1329-1333.
2. Fruehauf JP and Meyskens FL: Reactive oxygen species: A breath of life ordeath. Clin Cancer Res 2007; 13: 789–94.
3. Das PK and Kant R: Ethnobotanical studies of the tribal belt of Koraput (Orissa), Bull. medicoethno. bot. Res 1998; 9(3-4): 123-128.
4. Hingorani R, Deng J, Elia J, McIntyre C and Mittar D: Detection of Apoptosis using the BD Annexin V FITC Assay on the BD FACSVerse™ System. BD Biosciences 2011; 1-11.
5. Dawidowicz AL and Olszowy M: Mechanism change in estimating of antioxidant activity of phenolic compounds. Talanta 2012; 97: 312–7.
6. Berghe WM: Epgenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodelling of our epigenomes. Pharmacol Res 2012; 65: 565–76.
7. Cordell GA: Changing strategies in natural products chemistry. Phytochemistry 1995, 40: 1585-1612.
8. Benjamin NE: Measuring apoptosis in individual cells with the comet assay. Promegennotes 1997; 64: 13.
9. Rubinstein LV, Shoemaher RH, Paull KD, Simon RM, Tosmi S, Skehau P, Scudievo DA, Monks A and Boyd MR: Comparison of *in vitro* anticancer - drug-screening data queerated with terazolium assay-screening assay versus a protein assay against a diverse paued of human tumor cell tissues. JNCI 2003; 82(13): 113-118.
10. Galati G, Teng S, Moridani MY, Chan TS and Brien PJ: Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. Drug Metabolism and Drug Interaction 2000; 17: 311-349.
11. Jang HS, Kook SH, Son YO, Kim JG, Jeon YM, Jang YS, Choi KC, Kim J, Han SK, Lee KY, Park BK, Cho NP and Lee JC: Flavonoids purified from *Rhus verniciflua* stokes actively inhibit cell growth and induce apoptosis in human osteosarcoma cells. Biochim. Biophy. Acta 2005; 1726: 309-316.

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