

EVALUATION OF PHARMACOLOGICAL ACTIVITY AND LCMS ANALYSIS OF FLAVONOIDS IN COCONUT EMBRYO

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ABSTRACT

Among numerous species of plant growing in India, *Coccus Nucifera.L* (Coconut) occupies its unique place in the tradition, religions and cultures of different societies. However there are only a few studies undergone on coconut embryo. So, this study focussed on the phytochemical screening of methanol and chloroform extract of coconut embryo has revealed the presence of different phytoconstituents. The antiproliferative activity of coconut embryo was demonstrated using human colorectal adenocarcinoma (HT-29) cell line with MTT assay and confirmed by AO/EtBr. The methanol and chloroform extract of coconut embryo does not exhibit antibacterial activity. Antioxidant activity of methanolic extract of coconut embryo was recorded using DPPH assay. Therefore, the present study highlighted that the coconut embryo has found to be a potent nutritive source eventually it possess a significant antioxidant and anticancer activity.

KEYWORDS: Coconut Embryo, Extraction, Antimicrobial, Antioxidant, Cytotoxicity, MTT Assay, DPPH Assay, Cell Line, Flow cytometry.

1. INTRODUCTION

1.1 Coconut Embryo

Coconuts are well known in the vegetarian community as a trove of nutrition. Almost every part of the coconut is a resource. Coconut embryo is basically a soft, spongy formation that are found inside mature coconuts. Coconut embryo is an edible part of the coconut palm *Cocos nucifera.L* belongs to the family Arecaceae. Coconut embryo also known as coconut pearl, coconut apple, coconut bubble, sprouted coconut. The scientific term of coconut embryo is called “cotyledon”. Coconut embryo is well known for excellent sources of nutrients like proteins, vitamins like B, C, electrolytes, fibre, and few minerals like potassium, Phosphorus, calcium, manganese. The nutrient concentration of the embryo remains very high when consumed at the growth phase. Along with the essential nutrients, it is great in

maintaining human health. The chemical constituents of *C. nucifera* L. Have some of the biological effects, such as a anthelmintic, anti-inflammatory, antinociceptive, antioxidant, antitumor activity, anti-hypertensive, cardio-protective, anti-seizure, cytotoxicity, hepatoprotective, vasodilation, nephroprotective and anti-osteoporosis effects.



Figure 1: Coconut embryo.

Recently coconut Development Board of India has indicated coconut embryo used in treatment of heart related ailments and diabetes

1.2 Liquid Chromatography/Mass Spectrometry (LC-MS/MS)

Liquid Chromatography/Mass Spectrometry (LC/MS) is a powerful analytical technique that combines the resolving power of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography (LC) separates the sample components and then introduces them to the mass spectrometer (MS). The MS creates and detects charged ions. The LC/MS data may be used to provide information about the molecular weight, structure, identity and quantity of specific sample components.

LC-MS instruments are basically HPLC units with a mass spectrometry detector attached to it where as LC-MS/MS is HPLC with two mass spectrometry detectors. LC/MS is suitable for the analysis of large, polar, ionic, thermally unstable and volatile compounds. LC/MS eliminates the need for time-consuming chemical modifications.

High pressure liquid chromatography (HPLC) Liquid chromatography generally utilizes very small particles packed and operating at relatively high pressure, and is referred to as high performance liquid chromatography (HPLC). Modern LC-MS methods use HPLC instrumentation, essentially exclusively, for sample introduction.

In HPLC, the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase generally composed of irregularly or spherically shaped particles chosen or derivative to accomplish particular types of separations.

Mass Spectrometry (MS) Mass spectrometry is an analytical technique that measures the mass-to-charge ratio of charged particles. MS works by ionizing chemical compounds to generate charged and measuring their mass-to-charge ratios. In a typical MS procedure, a sample is loaded on to the MS instrument and undergoes vaporization. The components of the sample are ionized by one of a variety of methods which results in the formation of charged particles. The ions are separated according to their mass-to-charge ratio in an analyser by electromagnetic field.

1.3 Antimicrobial activity

There has been an increasing incidence of microbial infections in recent years, largely due to the increase in opportunistic fungal pathogens and the emergence of resistance microbial species. Although, fungal-related diseases may not be as common as other bacterial infections, when they are present, they could be difficult to eradicate, especially in immunosuppressive situations.

Mycotic infections have been observed to be the primary cause of mortality in patients with severely impaired immune mechanisms. Natural products from microorganisms have been the primary source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important because these may serve as promising sources of novel antibiotic prototypes.

Man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various disease. For example, the use of bearberry (*Arctostaphylosuva-ursi*) and cranberry juice (*Vacciniummacrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleucaalternifolia*) are described as broad-spectrum antimicrobial agents.

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods.

Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthesized molecules as potential antimicrobial agents. Pathogenic and food spoilage deterioration in both developed and developing countries.

There are several *in-vitro* methods used for evaluating anti-microbial activity, they are

1. Diffusion method

- Agar disk-diffusion method
- Antimicrobial gradient method
- Agar well diffusion method
- Agar plug diffusion method
- Cross streak method
- Poisoned food method

2. Dilution methods

- Broth dilution method
- Agar dilution method

3. Time-kill test (time-kill curve)

4. ATP bioluminescence assay

5. Flow cytofluorometric method

6. Agar disc diffusion method

Agar disc-diffusion testing developed in 1940,^[8] is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. It is also called as Kirby-Bauer test. This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, picks up moisture and the antibiotic diffuses radially outward through the agar medium producing an antibiotic concentration gradient. It is performed using Muller-Hinton Agar (MHA). It is a simple, more accurate, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided.

Minimum Inhibitory Concentration (MIC):

It is a lowest concentration of an antimicrobial agent that inhibits the visible in-vitro growth of microorganisms. It is expressed as mg/L or µg/µl.

Minimum Bactericidal Concentration (MBC):

It is the lowest concentration of antibiotic required to kill a particular bacteria.

1.4 Antioxidant activity

Free radicals are the type of reactive oxygen species (ROS) which include all highly reactive, oxygen containing molecules. They are capable of attacking healthy cells of the body, causing them to lose their structure and function, also involved in the oxidative damage of biological system. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative disease of aging such as cancer, diabetes mellitus, renal failure, cardio vascular disease etc.

To protect the cells and organ systems of the body against reactive oxygen species, human have evolved a complex antioxidant protection system. Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called “free-radical scavengers.”

The sources of antioxidants can be natural or artificial. Certain plant-based foods are thought to be rich in antioxidants. Plant-based antioxidants are a kind of phytonutrient, or plant-based nutrient. The body also produce some antioxidants, known as endogenous antioxidants. Antioxidants that come from outside the body are called exogenous. Antioxidants are said to help neutralize free radicals in our bodies, and this is thought to boost overall health.

Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidant that protect the body against deleterious effect. Oftentimes, amount of antioxidant present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore it is necessary to enrich our diet with antioxidant to protect against harmful disease. Antioxidants are the molecules that delay or prevent cell damage caused by free radicals. Antioxidants are used in food industry for preservation or protection against oxidative damage, to avoid deleterious changes and loss of commercial and nutritional value. There are several *in-vitro* methods for the estimation of antioxidant activity.

- DPPH Scavenging activity
- Hydrogen peroxide scavenging (H₂O₂) assay
- Nitric oxide scavenging activity
- Ferric reducing antioxidant power (FRAP) assay
- Thiobarbituric acid (TBA) method

➤ Ferric thiocyanate (FTC) method

DPPH Scavenging activity:

DPPH - 2,2-diphenyl-1-picryl-hydrazyl-hydrate

It was developed by Marasden Blois in 1958, to determine the antioxidant activity of single compounds and their mixtures. It is rapid, simple, accurate, inexpensive and widely used method. It is based on the spectrophotometric measurement of DPPH concentration changes resulting from the DPPH reaction with an antioxidant. Antioxidant analysis by other methods may be limited to those compounds soluble in the selected solvents. The advantage of this method is that DPPH is allowed to react with whole sample. This method may be utilized in aqueous and organic solvents and can be used to determine both hydrophilic and lipophilic antioxidants.

1.5 Anticancer activity

Cancer is the wide spread disease across all over the world It is the uncontrolled growth of abnormal cell anywhere in the body that invade or spread to nearby tissues. These abnormal cells are called cancer cells, malignant cells or tumor cells. The abnormal growth and diversion in observed in Cancer cell is caused by damage in these cells DNA. Genetic material inside cells that determine cellular characteristics and functioning. There are varieties of way that cellular DNA can become damaged and defective.

Any drug that is effective in the treatment of Malignant of cancerous disease is called anticancer drugs, current treatment include chemotherapy, radiotherapy and chemically derived drugs. But chemotherapy can cause lot of strain and further damage to the patients health. Herbal medicines are widely used for treatment of many diseases. It is used as primary source in many countries.

Many plant species are already involved in the prevention & treatment of Cancer. Many researchers are involved in identifying the plant properties that may give anti-cancer activity.

Compound that are extracted from plant which produced anti cancer activity are Polyphenols, brassinosteroids and taxols. Polyphenolic compound which includes flavonoids, tannins, curcumin, resveratrol and gallacatechin. These compounds are considered to have anticancer properties. Resveratrol can be found in food like peanuts, grapes and red wine. Gallacatechin is found in green tea. By intaking these kind of food in a health diet one can reduce the risk of Cancer because these compounds can act as good antioxidant.

Flavanoids are the secondary metabolites which are highly active compound. Many plants were Investigated for their flavanoid content and tested for their anticancer activity.

For example, Traditional chinese medicines like fern and letchi leaf considered to be consist of high content of flavanoids compounds like anthocyanin, flavones, flavonols, chalcone etc. It is identified that the fern of species *Dryopteris erythrosora* consist of flavonoids has the anticancer effect on human lung cancer cells (A456 cell line). Brassinosteroids are naturally occurring compounds found in plants to regulate growth, differentiation of cells, elongation of stem and root cell.

Cell lines

Cell lines are the cell cultures, cells can be obtained from tumors and subsequently cultured. They are cultured which proliferate in the given fresh medium and space. There are various cell lines used for various types of cancer obtained from particular type of cancer cells.

HT 29 is a type of cell line obtained from human colorectal tumor cells. This cell line was first established in 1964 by Jorgen fogh from the primary tumor of a 44-year old female with colorectal cancer. The cell lines are used not only for cancer research but also for identifying the biological activity. The cells are sensitive to drugs like 5-fluorouracil and oxaliplatin these are the standard for colorectal cancer treatment.

2. AIM AND OBJECTIVE

Coconut embryos are the latest food trend which taking the internet by storm. The coconut embryo is rich in antioxidant and nutrients. It is also a natural source of quick energy. It may also help to improve digestion and absorption of nutrients, vitamins and minerals, insulin secretion and restores thyroid function as well. It is the perfect snack for those who looking for weight loss and healthy snacks.

There is no research undergone for anticancer activity of coconut embryo. So, The main aim of the study was to analyze the photochemical Constituents of coconut embryo which is enriched with various antioxidants, anticancer properties. The various phytoconstituents makes the coconut embryo to be rich in nutritive.

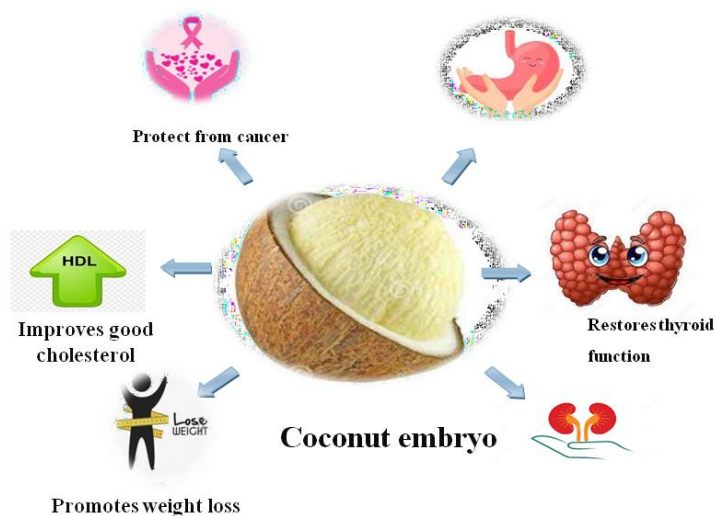


Figure 2: Health benefits of Coconut embryo.

3. MATERIALS AND METHODS

3.1 Sample Collection

The fresh coconut embryo of species *Cocos nucifera* L. was purchased from local markets in Tamil Nadu like Coimbatore, Erode. We got authentication for coconut embryo from Botanical Survey of India, Southern Regional Centre Tamil Nadu Agricultural University Campus, Lawley road, Coimbatore- 641003, Tamil Nadu.

3.2 Extraction

We have taken 3 germinated coconut and then the husk was removed. The embryo was collected from the germinated coconut without any contamination, then converted into small pieces and dried using hot air oven at 150°C until the

yellowish white colour changes into the brown colour. Dried sample was stored in air tight container.

From that we have taken 10gm of dried samples of coconut cotyledon in 100ml beaker then soaked with few ml of methanol and chloroform separately for 24 hours at normal room temperature. Soxhlet apparatus (hot percolation method) was used for the preparation of crude extract of methanol and chloroform. The temperature was set at 70°C and the apparatus was operated for 3 cycles then filtered by using whatmann filter paper. The extracts obtained from hot percolation were concentrated to obtain crude. The crude extracts were collected and maintained at room temperature for 48 hours.

3.3 LC-MS

LC-MS analysis of both methanol and chloroform extract of coconut embryo was carried out using waters xevo TQD with alliance 2695 equipped with an electro spray ionisation (ESI) ion source interface using symmetric C18 column (75×4.6 mm, 3.5µm). Mobile phase comprises of 0.1% formic acid (A) and 0.1% acetonitrile (B). The gradient was set up 10% B in 0mins, 10%B in 3mins, 98%B in 5mins, 98%B in 7mins, with a flow rate of 0.8ml/min using 10µl of sample injection volume. Column oven temperature was kept at 30°C. The mass analyses were done by positive mode and SCAN ion was selected. Electro spray ionization with positive ion polarity the capillary voltage was set to 5.02kV, the capillary temperature 300°C, the nebulizer pressure 40 psi and the drying gas flow rate 2ml/min.

3.4 Antibacterial Activity

Materials used

Bacterial species gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*K.pneumoniae* and *E.coli*), Muller Hinton agar, Himedia antibiotic disc(ciprofloxacin- std), nutrient broth.

Clinical microbial cultures and culture media

The methanol and chloroform extract of coconut embryo and ciprofloxacin (standard) In which the antibacterial property was examined against clinical *Bacillus subtilis*, *K. pneumoniae*, *E. coli*, *Staphylococcus aureus* Clinical Microbial cultures were procured from microbiology lab Coimbatore, Tamil Nadu. Muller-Hinton agar media of Himedia Pvt. Himedia sterile discs, Himedia antibiotics disc (Ciprofloxacin 5µg) Bombay, India were used for the microbial test. The antibacterial activity was evaluated by using the Himedia zone reader.

Inoculum's preparation

A 100µl clinical *Bacillus subtilis*, *K. Pneumonia*, *E. coli*, *Staphylococcus aureus* organisms were inoculated individually in 5.0 ml of sterile nutrient broth (NB) media and incubated at 37°C for 24hr. 200µl obtained from the 24hr fresh culture was dispensed into 30ml sterile nutrient broth and incubated for 2-4 hrs to standardize the bacterial culture to 10⁸ CFU/ml (colony forming units).

Disc preparation

The diluted formulation of chloroform and methanolic extract of Coconut embryo was added in Himedia sterile discs under aseptic conditions. Discs were air-dried completely under aseptic conditions and dried formulation discs were used for antibacterial activity.

Disc diffusion method

The antibacterial activity of chloroform and methanol extract of coconut embryo and Ciprofloxacin (5µg)) were evaluated separately using disc diffusion method. *Bacillus subtilis*, *K. pneumoniae*, *E.coli*, *Staphylococcus aureus* inoculums prepared using sterile nutrient broth media. Mueller Hinton agar double strength media were made by autoclaving 0.760 g in 100ml. Standardized inoculums (test microorganisms) inoculates the Muller Hinton agar plates using sterile cotton swabs. Chloroform and methanol extract of coconut embryo and Ciprofloxacin (50µg) discs were placed on agar plates under aseptic conditions separately. Agar plates were incubated at 37°C for 24hrs. Antibacterial activity was measured by using the Himedia zone reader.

3.5 Antioxidant (DPPH) Activity

Materials used

DPPH Reagent, Ascorbic acid (std), 96 well plate, ELISA Reader DPPH Scavenging activity The principle of this assay is based on electron transfer, i.e. the odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidant.

The sample stock solutions with 1.0 mg/ml strength were serially diluted to the resultant concentrations of 1000, 500, 250, 400 µg/ml in the coconut embryo of methanol and chloroform extract separately. 1 mL of 0.3 mM DPPH solution was added to 2.5 ml sample of various concentration and they were allowed to react at room temperature in dark place. Absorbance was calculated at 517 nm after 30 min using an ELISA reader, and they have changed the antioxidant potential percentage (AA) with the help of the following formula:

$$\text{Antioxidant percentage (I \%)} = \frac{\text{Abs. blank} - \text{Abs sample}}{\text{Abs. blank}} \times 100$$

3.6 Anticancer activity

Materials used

HT-29 (human colorectal adenocarcinoma) cell line, Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), streptomycin and penicillin, 96 well plate, phosphate buffer saline, acridine orange & ethidium bromide, fluorescence magnification microscope, ANNEXIN A5-FITC-Apoptosis detection kit.

Cell lines

HT-29 cell was procured from the national centre for cell sciences repository in Pune, India, for this study. The HT-29 (human colorectal adenocarcinoma) cell line was kept alive in Dulbecco's Modified Eagle Medium (DMEM) with a 10% Fetal Bovine Serum supplement (FBS). To prevent bacterial contamination, streptomycin (100 g/ml) and penicillin (100 U/ml) were added to the medium. The cell medium was kept at 37°C in a humidified 5 % CO₂ environment.

Cell culture and MTT Assay

The MTT assay was used to assess the cytotoxicity of the coconut embryo methanol extract in a 96-well plate. Using a laminar airflow cabinet, the entire operation was carried out aseptically. HT-29 cancer cells were seeded at 5 x 10⁴ cells per well in 96-well cells and incubated overnight. After 48 hours of treatment with coconut embryo methanol extract, the cells were extracted, washed with phosphate buffer saline (PBS), and then treated for 5 minutes at room temperature with acridine orange/ethidium bromide (AO/EB 1:1 ratio; 100 g/ml). The stained cells were examined

using a 40x fluorescence magnification microscope.

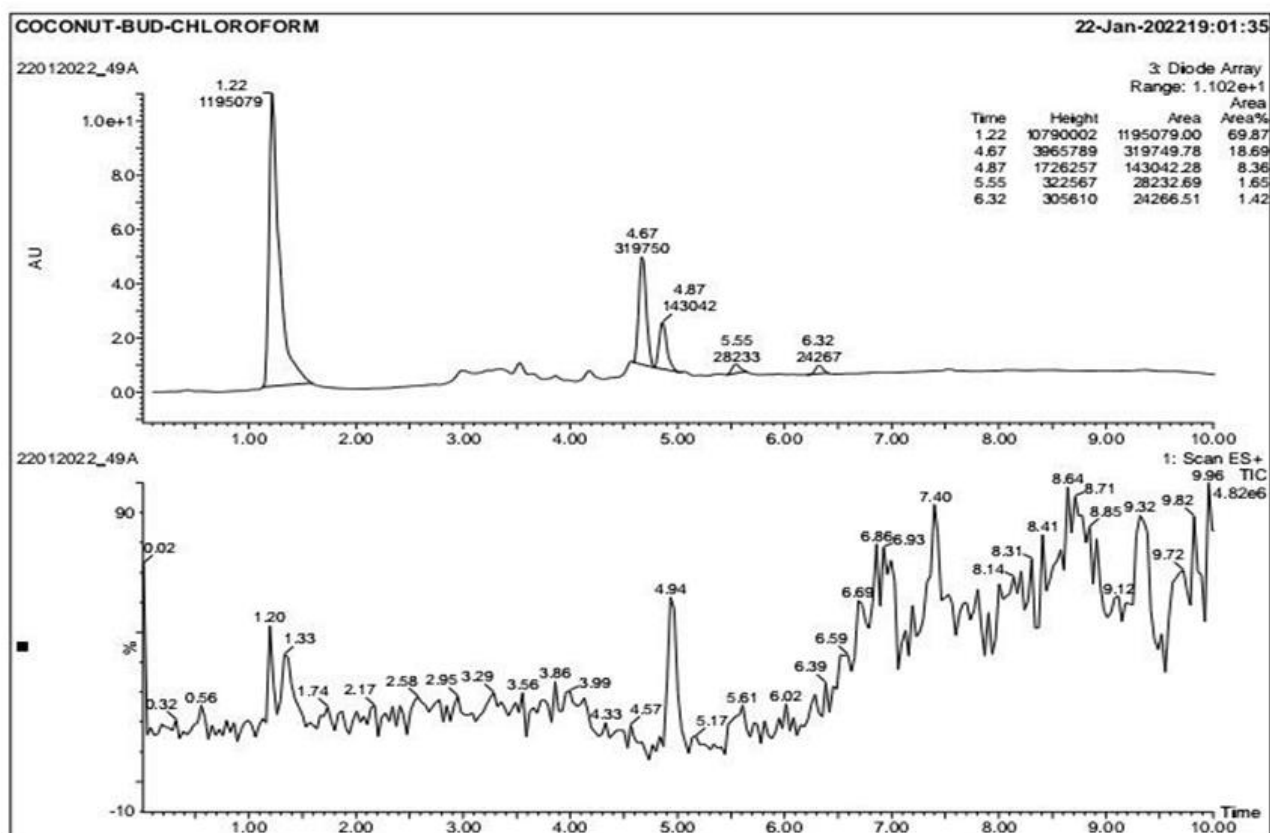
Flowcytometry analysis

Apoptosis assay

Apoptosis assay was carried out using ANNEXIN A5-FITC-Apoptosis detection kit by Beckman Coulter as per their manufacturing protocol. In brief, cell pellets from the control, silver nanoparticles treated groups were resuspended in 1x binding buffer to 5x10⁶ cells/ml, to which 1µL of annexin A5-FITC and 5µL of PI were added and incubated on ice for 15 min before being examined by fl\ cytometry.

4. RESULTS AND DISCUSSION

4.1 LC-MS Result



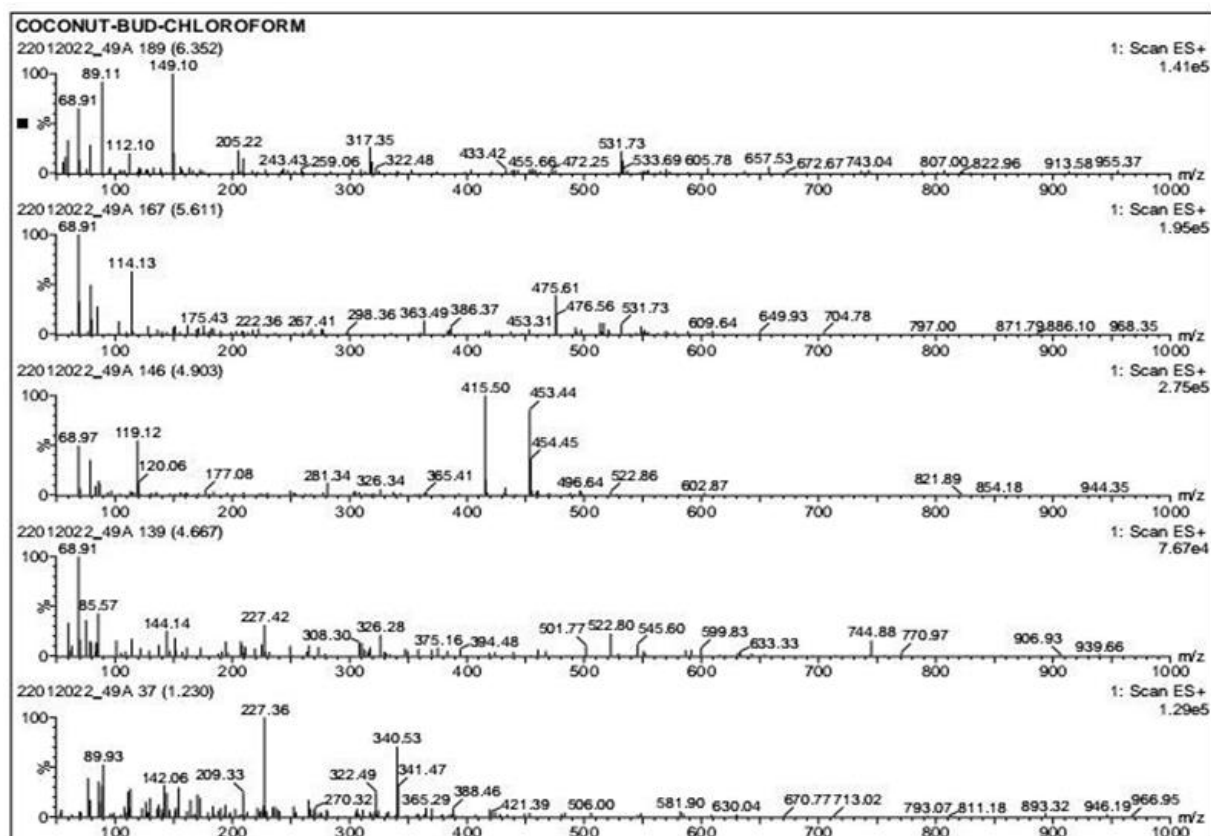
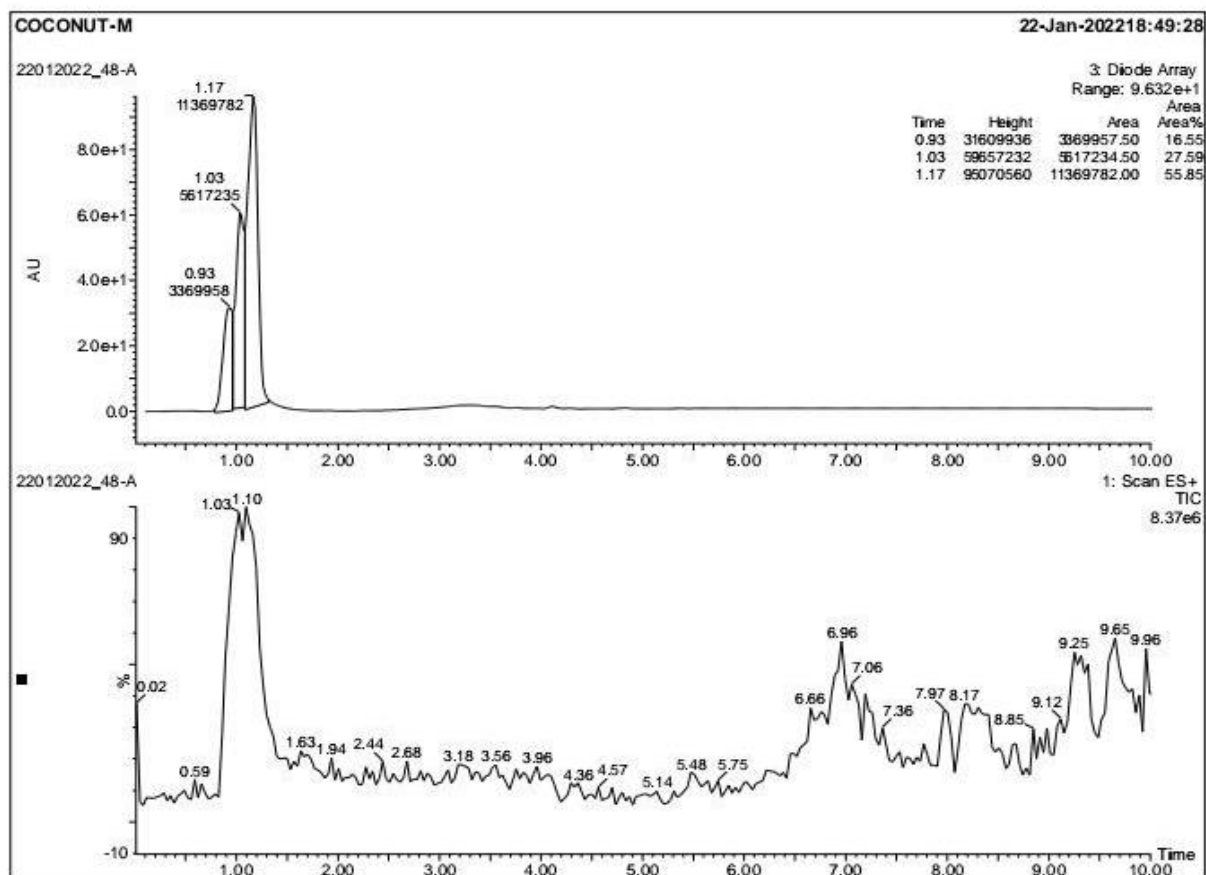


Figure: 4 LC MS chromatogram analysis of the chloroform extract of coconut embryo.



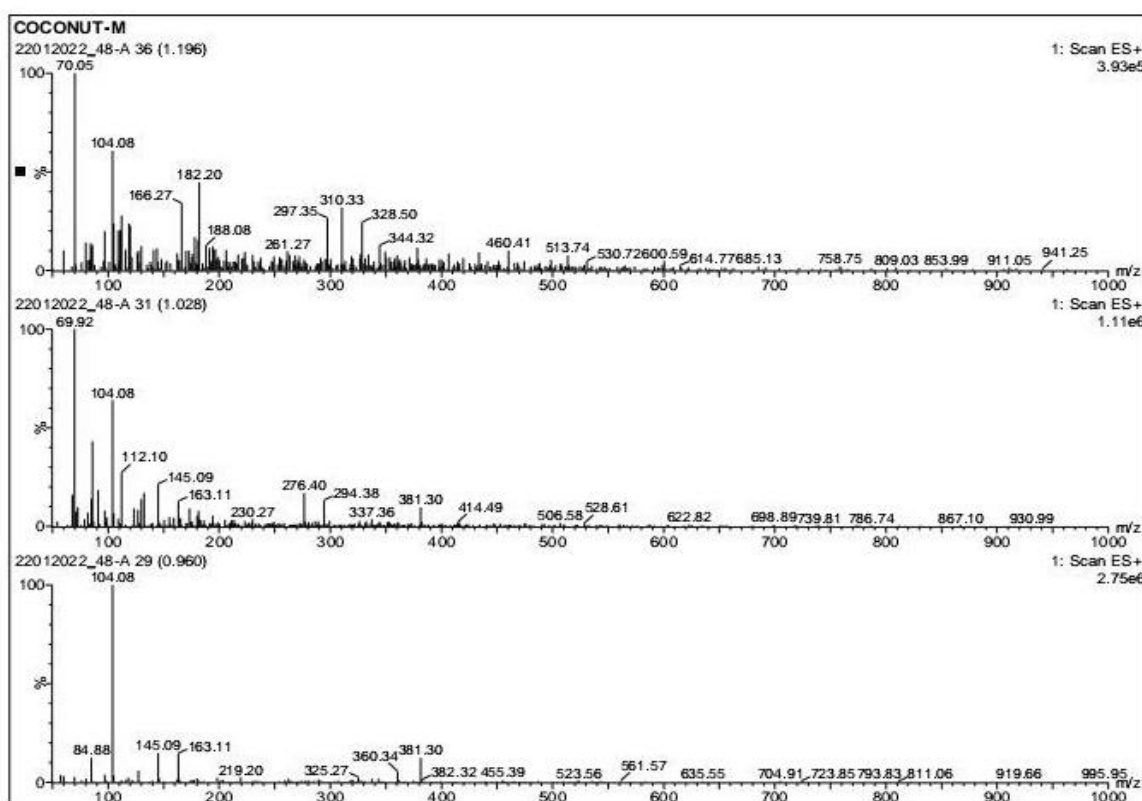


Figure 5: LC MS chromatogram analysis of the methanol extract of coconut embryo.

LC MS chromatogram analysis of the methanol and chloroform extract of coconut embryo showed many peaks which indicates the presence of 27 phytochemical constituents which is mentioned in the table. On comparison of mass spectra of the constituents with the standard mass value in pubchem database. Hydroquinone has similar peak in both methanol and chloroform extracts of coconut embryo.

Table 1: Phytoconstituents identified by LC-MS report.

S.NO	COMPOUND NAMES	MOLECULAR WEIGHT(m/z)	STD MOLECULAR WEIGHT
1	Flaone	365.29	365.4
2	Flavone	222.36	222.24
3	4.Flavanol	227.42	226.27
4	Flavoxate HCL	308.30	308.3
5	Myricetin	317.35	318.23
6	Apigenin	270.32	270.24
7	Baicalein	270.32	270.24
8	Genistein	270.32	270.24
9	Delphinidin	337.36	338.69
10	Petunidin	317.35	317.27
11	Dalbergin	267.41	268.26
12	Calophyllolide	415.50	416.5
13	Cardamonin	270.32	276.28
14	4-Hydrxoxy derricin	337.36	338.4
15	Spiro Pyrazolide 4-flavanone	506.00	506.6
16	Dimethoxyflavonone	298.36	298.29
17	Brequinar	375.16	375.4
18	Hydroquinone	112.10	110.11
19	3-Monodeutero flavanol	227.36	227.28
20	Beta-Sitosterol acetate	455.73	456.7

21	Phytol	294.38	296.5
22	Erucic acid	337.36	338.6
23	Vaccenic acid	281.34	282.5
24	Resveratrol	227.36	228.2
25	Myristic acid	227.42	228.37
26	Caffeoyl beta D glucose	341.47	342.3
27	5-p-Coumaroylquinic acid	337.36	338.31

4.2 ANTI MICROBIAL ACTIVITY

In the present study, Antibacterial activity was tested using agar disc diffusion methanol. The methanol and chloroform extract of coconut embryo were tested against selected gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacterial (*E. coli* and *K. pneumoniae*) species. Both the methanol and chloroform extract of coconut embryo does not showed significant concentration dependent antibacterial activity, expressed as zone of inhibition with respect to the above mentioned microorganisms. When compared to the standard drug ciprofloxacin, no effect was seen in both extract of coconut embryo.



Figure 6: Zone of inhibition on *K. pneumoniae*.



Figure 7: Zone of inhibition on *Bacillus subtilis*.



Figure 8: Zone of inhibition on *Staphylococcus aureus*.



Figure 9: Zone of inhibition on *E. coli*.

4.3 Antioxidant (DPPH) Activity

Antioxidant activity of methanol and chloroform extract of coconut embryo has been tested by DPPH radical procedure using ascorbic acid as a reference standard. The methanolic extract of coconut embryo showed nearly 73.3% of inhibition at the concentration of 500 μ /ml as compared to ascorbic acid (std), where the chloroform extract of coconut embryo does not showed any inhibition. The inhibition percent of DPPH radical at different concentrations of the methanolic extract is mentioned in the table 2.

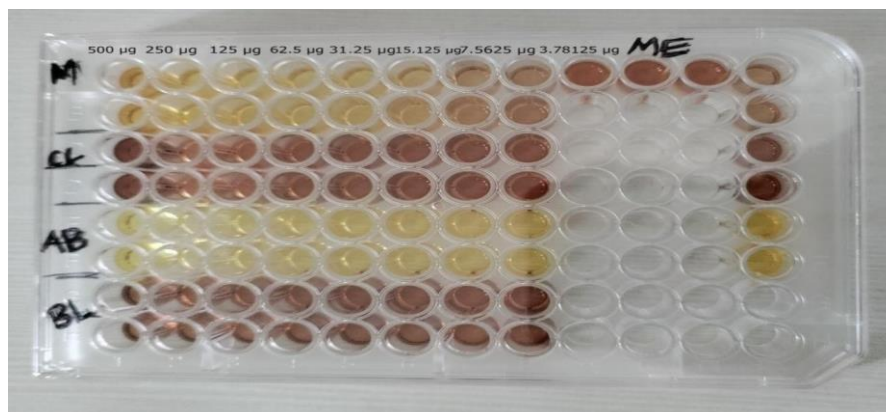


Figure 10: Antioxidant activity by 96 well plate.

Table 2: Inhibition percent of DPPH radical at different concentrations of the methanolic extract.

Concentration	OD @ 517nm	% Inhibition
500	0.116	73.33
250	0.143	67.12
125	0.162	62.75
62.5	0.186	57.24
31.25	0.210	51.72
15.125	0.231	46.89
7.5625	0.261	40.00
3.78125	0.267	38.62
Ascorbic acid (100)	0.126	71.03

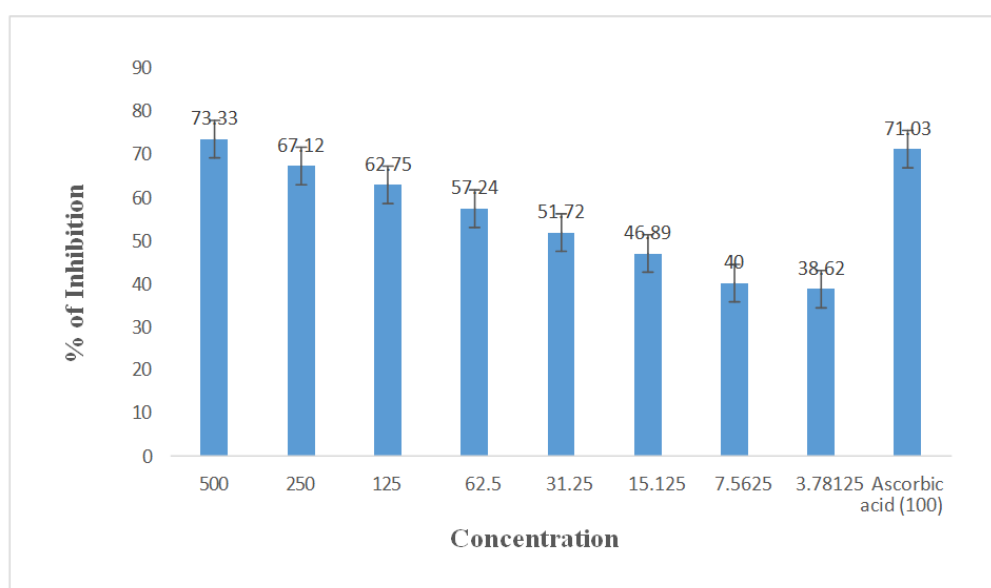


Figure 11: Graph plotted against Concentration and percentage of inhibition.

4.4 ANTICANCER ACTIVITY

HT-29 cells treated with in control and coconut embryo methanol extract 0 to 500 µg/ml at 24hrs, stained with dual dye AO/EB and then analyzed by fluorescence microscopy. Living cells normal green nucleus appeared, early apoptotic condensed or fragmented form of yellow color nucleus with chromatin, late apoptotic chromatin condensation or fragmentation orange-stained nuclei and necrotic cells.

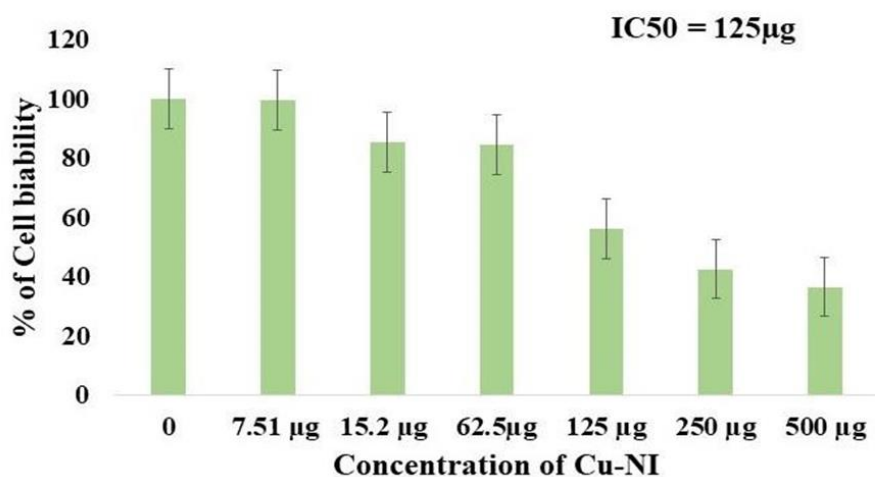


Figure 12: Cytotoxic activity of coconut embryo extract methanol against HT-29 cells.

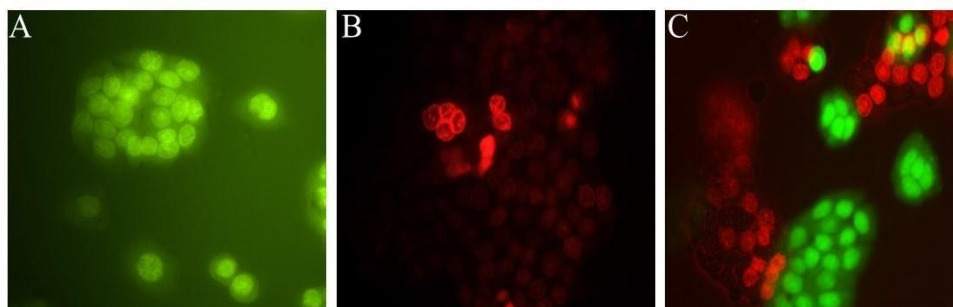


Figure 13: Cytotoxic activity of coconut embryo methanol extract against HT-29 cells. (A): Untreated, (B): Treated, (C): Merged.

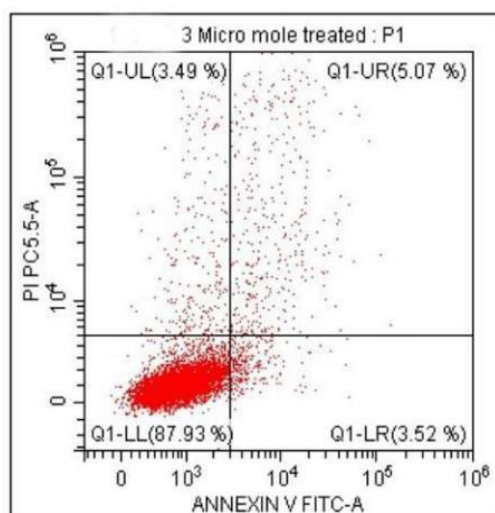


Figure 14 represents the typical fluorescence-activated cell sorting (FACS) photomicrograph. The statistical analysis of early and late apoptotic cells was determined by (FACS) as shown in Figure 14. The cell histogram revealed cytotoxic effects of chloroform extract 3 micromolar concentrations. The methanol extract induced moderate toxicity towards HT-29 cells. Higher concentration of AuNPs the early and late apoptosis rate was 3.49% and 5.07%, respectively.

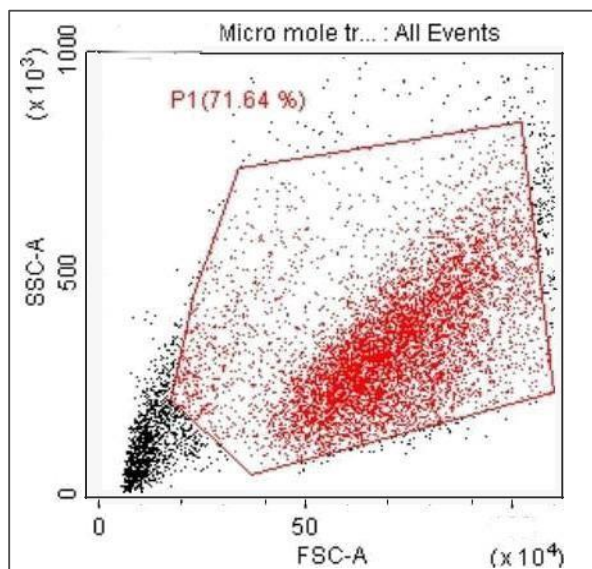


Figure 14: The fluorescence scatter image after treated chloroform extract (3 µg/ml) to HT-29 cell lines.

5. CONCLUSION

To the best of our knowledge this is the first report of methanol and chloroform extract of coconut embryo to determine the antioxidant and anticancer properties. This present study possesses the predominant antioxidant activity and significant reduction in cancer cell line proliferation in methanolic extract. The chloroform extract does not showed any activity This study highlighted the presence of flavonoids content in coconut embryo using hyphenated technique LC MS in both methanol and chloroform extract. In addition this study proves that coconut embryo does not possess any antibacterial activity in both methanol and chloroform extract. These findings showed that the coconut embryo is rich in many health benefits. This research will help us to rise the herbal drug development to the next level which would relieve human afflictions.

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