RESEARCH PAPER

SYNERGISTIC ANTIOXIDANT AND NON-TOXIC ANTICANCER POTENTIAL OF *MOMORDICA CHARANTIA* AND *DAUCUS CAROTA*: A FREE RADICAL SCAVENGING APPROACH

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ABSTRACT

Due to their potential therapeutic uses and health benefits, the hunt for natural antioxidants has attracted a lot of attention. Phytochemical compounds derived from plants have garnered a lot of attention due to their potential health benefits and therapeutic qualities. In this study, we conducted a comprehensive phytochemical screening of two plant species, *Momordica charantia* (commonly known as Bitter gourd) and *Daucus carota* (also known as Carrot). The investigation exposed the presence of diverse bioactive compounds, particularly flavonoids, phenols and tannins, which contributes to free radical scavenging activity.

Both extracts were evaluated for phytochemical screening, total phenolic content and total flavonoid content, IR spectroscopy of active constituents and free radical scavenging activity using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method with ascorbic acid as the reference standard.

The results showed that both *Momordica charantia* and *Daucus carota* are potent and showing promising free radical scavenging property. It was observed that *Momordica charantia* potentiate the antioxidant activity of *Daucus carota*. This synergistic action is used as an effective and alternative for the treatment of cancer due to their free radical scavenging activity. In addition, both the extracts showed non-toxic effects against a diseased human lung fibroblast (COPD), HCC7231 (TACC CCL-96) cell lines.

These extracts not only prevent carcinogenesis but can also serve as adjuvant approach in cancer management.

Keywords: *Momordica charantia, Daucus carota*, phytochemical screening, free radical scavenging activity, DPPH method, Synergistic action

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INTRODUCTION

The carrot (*Daucus carota L.*) is a widely cultivated root vegetable belonging to the family Apiaceae, also known as the *Umbelliferae*. It is a biennial plant primarily grown for its fleshy, edible taproot, which is most commonly orange but also occurs in purple, red, yellow, and white varieties. Carrot is native to Europe and southwestern Asia but is now grown worldwide due to its rich

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nutritional profile and versatility in culinary and medicinal uses [1].

Carrots are known for their beta-carotene, a predecessor of vitamin A, which is vital for vision, immune function, and skin health. Vitamin C helps in collagen production. Collagen is a protein that maintains elasticity of skin and delays the ageing process and wrinkle formation.

It contains antioxidants such as polyphenols and flavonoids. Polyphenols mainly contains derivatives of hydroxycinnamic acids. Chlorogenic acid is a major constituent representing 42.2% to 61.8% of total phenolic compounds [2]. These compounds contribute for the therapy of various diseases, including cardiovascular disorders [4], certain cancers [2] and age-related macular degeneration.

In traditional and modern medicine, carrots have been used to treat digestive problems, boost immunity, and improve skin health. There juice is rich in antioxidants and often recommended for detoxification and liver support [3].

Carrot seed oil is used in aromatherapy and skincare for its anti-inflammatory [4] and rejuvenating properties. In herbal treatment, carrot leaves are used. An infusion of the leaves and seeds act as a uterine stimulant during childbirth. These leaves, along with roots or seeds, are applied as a plaster for oozing wounds and ulcers[5].

Figure 1: Carrot and Carrot seed

The bitter gourd (*Mordica charantia*) is tropical and subtropical vines belong to Cucurbitaceae family. Because of its nutritional and therapeutic qualities, bitter gourd, which is inhabitant to Asia and parts of Africa, is now grown all over the world [6].

Bitter gourd is well known in the medical community for its strong antiviral [7], anti-inflammatory [8], antifungal [9], antioxidant [10], and anti-diabetic effects [11]. It is useful in both conventional and contemporary diabetes treatments because it contains bioactive substances like momordicin, charantin, vicine, and polypeptide-p that have been demonstrated to help control blood sugar levels. Additionally, in Chinese and ayurvedic medicine used to treat liver problems, and digestive issues.[12]

Figure 2: Bitter Gourd

Oxidative stress is a detrimental imbalance between the body's capacity to use antioxidants to counteract reactive oxygen species, or free radicals, and their production.

ROS can destruct and modify DNA, disrupt cell signalling, and increase inflammation. All these factors can let cancer cells grow and multiply. However, researchers have looked into oxidative stress as a possible treatment since tumour cells also depend for their development and continued existence [13].

Excessive ROS can damage vital cellular components leading to conditions such as amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease [14]. Chronic inflammation, which can cause oxidative stress[15], is common in diseases like rheumatoid arthritis, inflammatory bowel disease, and chronic obstructive pulmonary disease (COPD).

A common technique for assessing the antioxidant activity is the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. The DPPH assay measures the oxidative stress reduction using the DPPH free radical as a probe. Ascorbic acid is used as the reference standard [16].

Further antioxidative events were correlated and evaluated by using on cell lines i.e., diseased human lung fibroblast (COPD), HCC7231 (TACC CCL-96) using lenvatinib, as positive control.

MATERIALS AND METHODS

Chemicals:
Analytical grade 1, 1-diphenyl-2-picryl-hydrazil (DPPH), ascorbic acid and methanol are from Sigma Aldrich Ltd [28].

Plant Materials: Carrot and Bitter gourd were grated and finely chopped respectively and air dried.[29-30]

Methods
Several techniques have been used for the extraction of carrot and bitter gourd which are given below

Maceration, Soxhlet extraction, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, cold pressing, and enzyme-assisted extraction are some of the methods used to extract carrot and bitter gourd.[31]

Maceration Method [32-35]
Using the maceration method dried and ground bitter gourd and carrot, is put in a container with methanol. For 24 to 72 hours, the mixture is maintained at room temperature with sporadic shaking or stirring. The concentrated extract is obtained by filtering the solution and evaporating the solvent after the extraction period.

Procedure of Maceration

Plant Material Preparation
Gather and thoroughly dry the plant material. To improve extraction, crush or grind it into powder or tiny pieces.

Soaking in solvent
Take the plant material in an extraction vessel or sanitized container. Depending on the compound of interest, add enough solvent (such as ethanol, methanol, water, or another substance) to completely submerge the plant material.

Mixing
To guarantee that the solvent evenly penetrates the plant material, gently stir or shake the mixture. Allow the mixture to stand at room temperature or a regulated temperature for a predetermined amount of time, typically 24 to 72 hours, and cover the container to prevent solvent evaporation.

Filtration
To isolate the extract from the residue, pass the mixture through filter paper or a fine mesh screen after the maceration period.

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Concentration
If necessary, the filtrate (liquid extract) can be made more concentrated by heating it gently or by letting the solvent evaporate under low pressure.

Storage
To maintain the extract's qualities, keep it out of direct sunlight and heat in a sterile, airtight container.

Phytonutrient screening [36] of DC and MC
Phytonutrient screening of DC & MC for the presence of proteins, alkaloids, flavonoids, phenols & tannins was carried out.

Test for alkaloids
0.4 g of DC & MC was mixed with 10 ml of 2% HCl and warmed and filter and filtrate was treated with potassium bismuth (Dragendroff's reagent). Formation of orange or reddish-brown precipitate verifies the existence of alkaloids. This method is simple and reliable.

Test for flavonoids
50 mg of DC & MC was soaked in distilled water and filter. Take 10 ml of filtrate and add 5 ml of lead acetate solution. The yellow or pale-yellow precipitate confirms flavonoids. The color is due to formation of insoluble complex of extract with lead ions.

Test for phenols & tannins
50 mg of DC & MC was boiled in distilled water and filter. To the filtrate add 2ml of dilute NH_3, reddish-yellow color indicates phenolic compound.

Test for proteins
To 1ml of DC & MC, add mixture of copper sulphate and sodium hydroxide. The violet or purple coloration due to formation of complex between copper ions and peptide bonds shows the existence of proteins.

Total phenolic content (TPC) [37]
Phenolic compounds are key components of plants that have red-ox properties, which contribute to their free radical reduction, the group responsible is hydroxyl.
The Folin–Ciocalteu reagent, a mixture of phosphomolybdic and phosphor tungstic acids was used to determine the phenolic content. The results were reported in gallic acid equivalents (GAE) per gram dry extract weight and were derived from a calibration curve of gallic acid (0–250 µg/mL) (y = 9.53x − 0.13, R2 = 0.996). TPC were calculated in gallic acid equivalent (GAE) milligrams per gram. 500 lL of Folin–Ciocalteu reagent (10%) was mixed with a standard solution of gallic acid (0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL) to make the calibration curve.

The 200 lL aliquots of each of the three individuals were then analysed. 1500 lL of 7.5% Na_2CO_3 was used to initiate the reaction and allowed for 30 min at 25 ^{0}C. The double beam UV–Visible spectrophotometer (Shimadzu UV-1700, Japan) used to document absorbance at 765nm.

Total flavonoid assay (TFC) [38]
The TFC were estimated by colorimetric method with $AlCl_3$, using standard quercetin. 1mL of individual and combined extracts of DC & MC or standard quercetin (0.005, 0.01, 0.02, 0.03 and0.04 mg/mL) was reacted with 1 mL of $AlCl_3$ (2 %) and sodium acetate and incubated for 1 h, Absorbance was calculated at 420 nm. Total flavonoid contents were calculated as milligram of quercetin equivalent (QE)/g from the calibration curve: y = 6.406 x 0.012 (R2 = 0.993).

IR Spectroscopy
The IR spectrum, which ranges from approximately 4000 to 400 cm^{-1}, was recorded using IR-Spirit-L.

Free radical inhibition activity by DPPH assay method [39-40]
DPPH (1, 1-diphenyl-2-picryl-hydrazil) is commonly used spectrophotometric method for measuring antioxidant activity using methanol as solvent. DPPH is constant free radical with deep violet color in solution. The unpaired electron on nitrogen is responsible for the color.
The main principle is antioxidants can donate H atom and neutralizes DPPH radical to 1, 1-diphenyl-2-picryl hydrazine and color fades. The extent of discoloration is directly proportional antioxidant activity and absorbance measured at 517 nm.

Chemicals used
Analytical grade 1, 1-diphenyl-2-picryl-hydrazil (DPPH), ascorbic acid (AA) and methanol are from Sigma Aldrich Ltd [28].

Preparation of DPPH solution: Dissolve 33 mg of DPPH in 1L methanol and keep in dark to protect from sunlight, as DPPH is light sensitive.

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Sample preparation

Preparation of stock solution of extracts

Prepared by dissolving the extract of DC, MC and DC + MC in 100 ml of methanol

Standard preparation

Preparation of standard solution

Dissolve 50 mg of ascorbic acid in 100 ml of methanol

Procedure

Prepare 10, 20, 30, 40, 50 µg/ml concentrations of extracts and AA, add 1 ml of DPPH (0.1mM dissolved in methanol) mix well and kept in dark for 30 minutes. Absorbance was measured against blank (DPPH + methanol) at 517 nm.

Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \{(A0 - A1)/A0\} \times 100\%$$

Where A_0 = absorbance of the control, and A_1 = absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and IC_{50} was calculated.

***In Vitro* Screening for Anti Lung Cancer Activity** [41]

Human lung fibroblast (COPD), HCC7231 (TACC CCL-96) cells were preserved at 37ºC in an incubator with a humidified atmosphere of 5% CO_2 / 95% O_2. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, sigma Aldrich, India) containing 5% fetal bovine serum (FBS, Sigma Aldrich, India), 100 mg/ml of streptomycin, 100 unit/ml of phenoxy methyl penicillin by Sigma Aldrich, India and 2 mM L-glutamine (Sigma Aldrich, India).

MTT Assay [42]

To evaluate the chemotherapeutic effect of **MC, DC, MC+DC** on Human lung fibroblast (COPD), HCC7231 (TACC CCL-96) cell viability, the MTT assay was conducted following previously established protocols. Briefly, cells were seeded in 96-well tissue culture plates at a density of 4×10^4 cells/well and incubated for 48 h at 37 °C. Following incubation, cells were treated with these extracts, lenvatinib (positive control for an additional 48 h.

Post-treatment, the culture medium was removed, and MTT solution (0.5 mg/mL) was added to each well and incubated for 2 h at 37 °C in a CO_2 incubator. Supernatant was then removed and DMSO was added to dissolve the MTT formazan. Finally, the absorbance of developed purple colour was read at 570 nm using a microplate reader. The cytotoxicity values were evaluated using absorbance values implementing formula:

$$\% \text{ Cytotoxicity} = (A_{control} - A_{sample}) / (A_{control}) \times 100$$

Where, A_{sample} is the absorbance of sample and $A_{control}$ is the absorbance of control.

The half-maximal inhibitory concentration (IC_{50}) was determined from dose-response curves to quantify the efficacy of MC, DC, MC+DC in reducing cell viability.

Statistical Analysis [42]

Cell viability data were obtained from four independent experiments. Statistical analyses were conducted using Graph Pad Prism version 7. A two-way ANOVA followed by Dunnett's post hoc test was employed to assess statistical significance relative to the control group. Data are presented as mean ± standard error of the mean (SEM). Statistical significance was denoted as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). "N.S." indicates results that were not statistically significant.

RESULT AND DISCUSSION

The antioxidant activity entails neutralizing harmful free radicals that can harm cells and lead to aging and various diseases. Antioxidants aid in safeguarding and stabilizing these reactive molecules and lowering oxidative stress. Phytochemical analysis was performed on *Daucus carota* (DC) and *Momordica charantia* (MC). This analysis aimed to categorize the existence of different bioactive compounds. The findings enhanced our perceptive uses of DC and MC in creating natural oxidative stress reduction-based remedial approaches.

Both extracts are rich in polyphenols and flavonoids, demonstrating significant antioxidant properties, which contribute to various protective and disease-fighting capabilities.

The current learning designed to assess the potential of DC and MC through phytonutrient screening, utilizing methods to determine TPC, TFC and the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. The study was planned

to get conclude extracts with potent oxidative stress reduction activity.

The DC and MC were air dried, and the bioactive compounds were extracted through a maceration method using methanol as a solvent. The resulting extracts were then used to evaluate their capacity for free radical inhibition. Phytochemical analysis showed that both DC and MC contained phenols, flavonoids, and tannins, suggesting their potential as natural antioxidant sources. The amounts of TPC & TFC have been shown to have a positive association with oxidative stress reduction.

IR-Spirit-L spectrophotometer was used to record the infrared spectra between 4000 and 400 cm^{-1}. A hydraulic press was used to compress the sample into a pellet after it had been finely powdered with dry KBr. After that, the KBr pellet was put in the sample container, and the spectrum was acquired using normal procedures. IR spectrum of DC shows the broad peak at 3370 cm-1, (–OH group), 2930 cm-1 (Ar C-H), 1726cm-1 (Ar C=C) and 1633 cm-1 (C=O), may be **Chlorogenic acid (Figure 3)**. Similarly, spectrum of bitter gourd extract shows the broad peak at 3384 cm-1 (–OH), 2927 cm-1 and 2855 cm-1 (Aliphatic C-H), 1711cm-1 (Ar C=C) and 1602 cm-1 (C=O), may be **Momordicine-I (Figure 4).**

Total phenolic content at 0.1 mg/ml showed 34.57 ± 0.80, 33.03 ± 0.74 and 38.72 ± 0.62 Total flavonoid content at 0.04 mg/ml showed 26.6 ± 0.60, 27.94 ± 0.23 and 28.6± 0.46 for DC, MC and DC+MC respectively. (**Table 2**)

The DC, MC and DC+MC indicate natural antioxidant potential. DC demonstrated slightly elevated levels of phenols, flavonoids and tannins. Other elements, such as the presence of additional active components or synergistic interactions among various compounds, could also play a role in the observed similar effectiveness of DC, MC and DC + MC extracts in neutralizing liberated radicals.

In DPPH assay, both extract and combination displayed the ROS quenching against DPPH radicals. The % inhibition was measured at 10, 20, 30, 40 and 50μg/ml. Unexpectedly the results proved that both DC and MC extracts exhibited promising action as that of positive control AA with 0.8 absorbance **(Figure 5).**

The IC_{50} values revealed that, DC (0.78±0.042), MC (0.76±0.022) and DC+MC (0.79±0.042) and AA (0.8±0.10) **(Figure 6).** The values were dose dependent and % scavenging activity of DC was found to be 56.69%, MC 81.32% and DC+MC 93.61% **(Figure 7).**

Table 1: Phytochemical tests for DC and MC

In addition, the MTT assay results for compounds DC, MC and DC+MC at 72h on Human lung fibroblast (COPD), HCC7231 (TACC CCL-96) cell lines showed IC_{50} values of **185 ± 16.63 nM, 189 ±8.62 nM, and 190 ± 16.00 nM**, respectively. These values are comparable to the IC_{50} of the standard drug Lenvatinib**,** which was found to be **186 ±6.04 nM (Table 3)**. The corresponding cell viability for compounds DC, MC and DC+MC was observed to be **86.8 %, 88.9 %, and 87.1 % (Fig 8)** respectively, which further supports their potent anti-lung cancer activity. The compounds were comparably superior to the standard Lenvatinib**).**

In the current research, the values for phenolic content showed minor differences from those found in existing literature. Such discrepancies may be linked to differing levels of sugars, carotenoids, AA, extraction techniques, duration, or the geographical factors that affect phenol levels. The results indicated that each extract individually contained a considerable amount of phenols and flavonoids, while their combined form demonstrated the greatest antioxidant activity. This suggests that natural food ingredients may work better together. It supports using them in combination as dietary supplements and functional foods.

CONCLUISON

The current research emphasizes that various herbs contain distinct bioactive compounds with differing antioxidant capacities, and their combination can lead to either increased or decreased activity based on how they interact—whether synergistically, additively, or antagonistically. In this study, the synergistic interaction identified between *Daucus carota (DC)* and *Momordica charantia (MC)* significantly improved their antioxidant and therapeutic effectiveness. Their strong ability to scavenge free radicals indicates promising possibilities for cancer prevention and treatment. Ongoing exploration of these extracts supports their potential as natural anticancer agents, aiding in future progress in cancer research and treatment methodologies.

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PHYTO CONSTITUTES	*DAUCUS CAROTA*	MOMORDICA CHARANTIA
Carbohydrates	+	-
Proteins	-	-
Phenols and Tannins	+	+
Flavonoids	+	+
Alkaloids	-	+

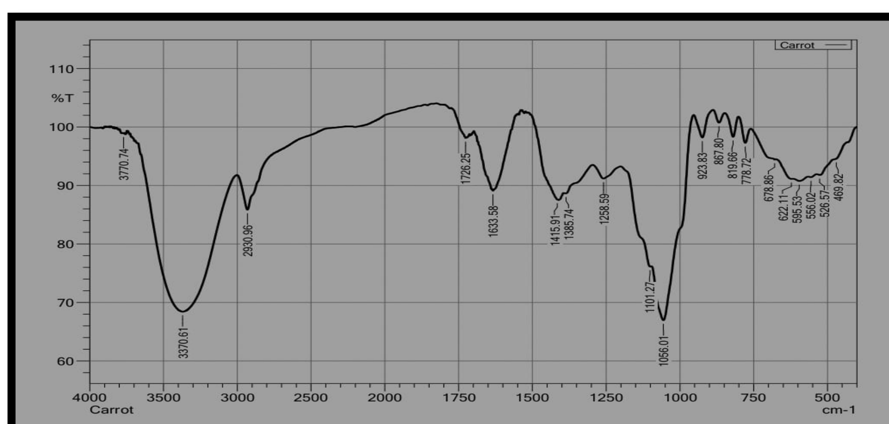
Table 2 Total phenolic and flavonoid contents of DC and MC and DC+MC combination

Sample	Phenolic content (mg GAE/g)	Flavonoid content (mg QE/g)
DC	35.47 ± 0.08	25.5 ± 0.06
MC	32.03 ± 0.47	27.49 ± 0.32
DC+MC	37.27 ± 0.26	28.58± 0.64

Values are expressed as mean ± standard deviation (n = 3). Values are statistically different at $p<0.05$

Figure 3: Infrared spectral study of DC

The IR spectrum of carrot extract shows the broad peak at 3370 cm-1, (–OH group), 2930 cm-1 (Ar C-H), 1726cm-1 (Ar C=C) and 1633 cm-1 (C=O), may be **chlorogenic acid**



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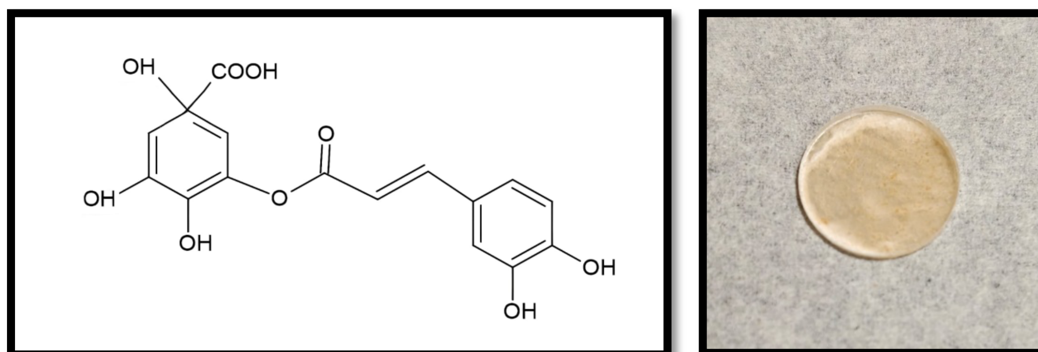
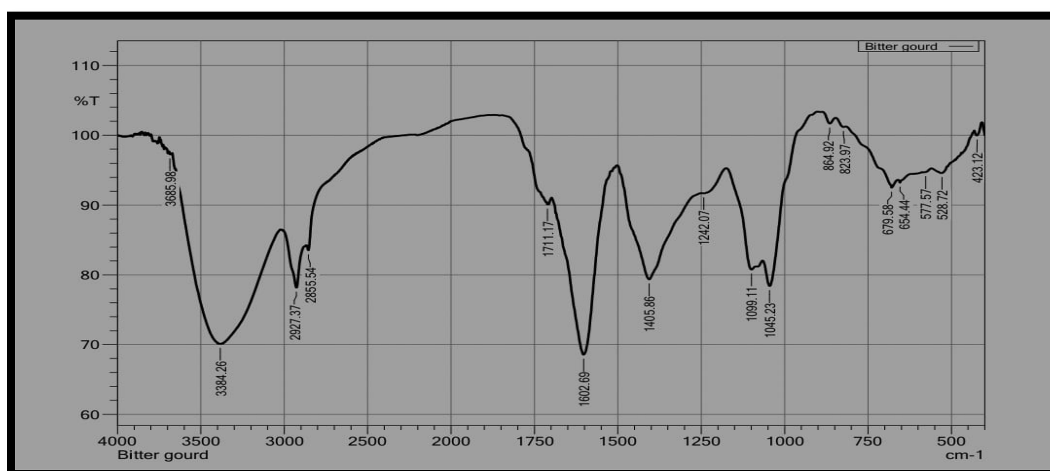


Figure 4: Infrared spectral study of MC

The IR spectrum of bitter gourd extract shows the broad peak at 3384 cm-1 (–OH), 2927 cm-1 and 2855 cm-1 (Aliphatic C-H), 1711cm-1 (Ar C=C) and 1602 cm-1 (C=O), may be **Momordicine-I.**



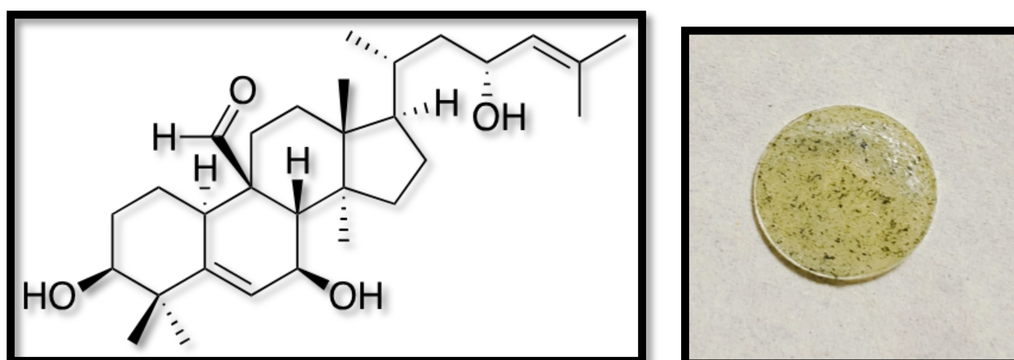


Figure 5: Free radical scavenging activity of DC, MC and MC+DC using DPPH

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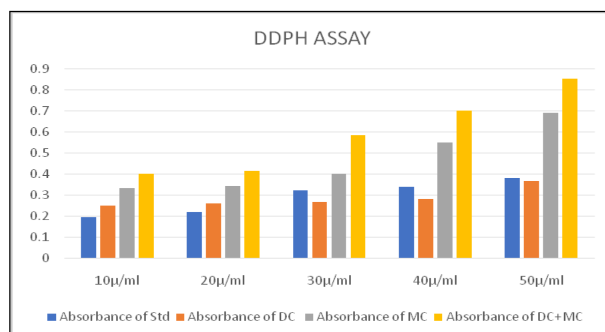
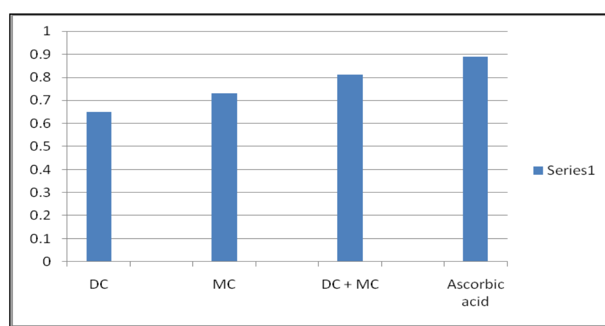


Figure 6: IC_{50} values in mg/ml for DPPH radical scavenging activity



X-axis-Concentration of oils in mg/mL Y-axis-DPPH radical scavenging

Figure 7: % scavenging activity of DC, MC and MC+DC using DPPH

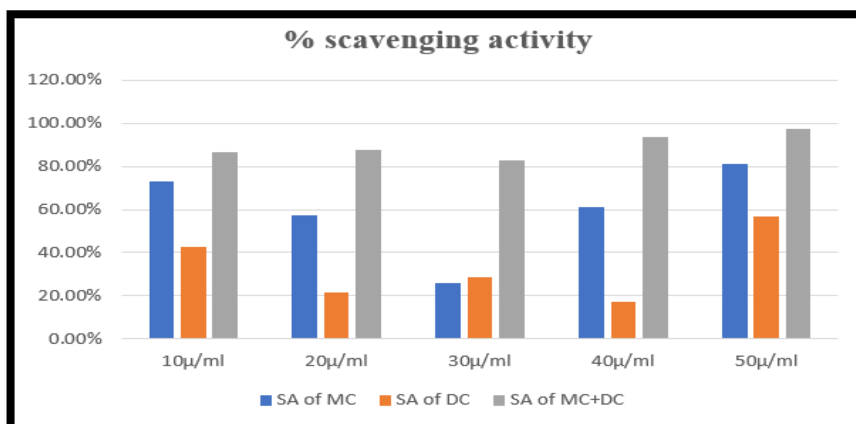


Table 3: Cytotoxic effect and cell viability of **DC, MC, DC+MC** was readably determined against diseased Human lung fibroblast (COPD), HCC7231 (TACC CCL-96) Cell Lines by MTT assay, time 72h.

Sl.no	Compound Code	IC50±SEM (nm)
1	**DC**	185±16.63
2	**MC**	189±8.62
3	**DC+MC**	190±16

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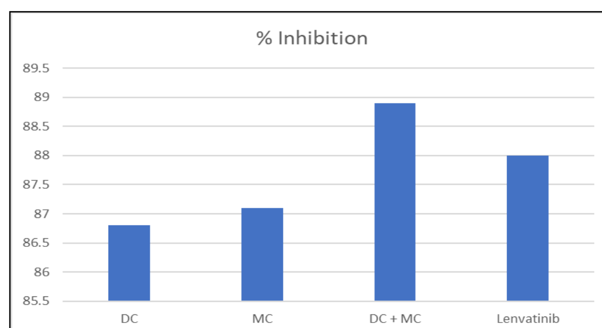
4	**Lenvatinib**	186 ±6.04

Fig 8: Percentage of Lung cancer cell line Human lung fibroblast (COPD), HCC7231 (TACC CCL-96) that inhibited by **DC, MC, DC+MC** by performing MTT Assay



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