Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

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Research Article

Comparative Study of Ethanolic and n-Hexane Extracts of Cassia angustifolia as Anticancer Agents against Human Cancer Line MDA-MB-231

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ABSTRACT

Cancer is characterized by the uncontrolled and undifferentiated proliferation of cells, with the potential to invade and metastasize to various parts of the body. The use of conventional herbal extracts represents a promising approach in the search for novel anticancer agents due to the presence of bioactive phytochemicals in medicinal plants. In this study, the anticancer activity of ethanolic and n-hexane extracts of *Cassia angustifolia* was evaluated against the human breast cancer cell line MDA-MB-231. Five different concentrations (0, 50, 100, 250 and 500 µl/ml) of each extract were tested to determine the optimal cytotoxic dose. At 250 µl/ml of the ethanolic extract, most cells appeared rounded and detached from the surface, while the remaining cells were elongated and loosely adhered. At the highest dose of 500 µl/ml, extensive cell death was observed, with cellular debris indicating significant cytotoxicity. In contrast, the n-hexane extract at 50 µl/ml showed negligible changes in cell morphology compared to the control. However, increasing concentrations led to reduced cell viability. The half-maximal inhibitory concentration (IC_{50}) was determined to be 83 for the ethanolic extract and 283 for the n-hexane extract. These findings suggest that the ethanolic extract of *Cassia angustifolia* exhibits greater anticancer efficacy against the MDA-MB-231 cell line compared to the n-hexane extract, likely due to the differential solubility and availability of bioactive compounds in the two solvents.

Keywords: Anticancer, Breast cancer, Plant extract, Cassia angustifolia, Phytochemicals.

OPEN ACCESS

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Article History
Received: March 02,2025
Accepted: August 22, 2025
Published: September 04, 2025

INTRODUCTION

Medicinal plants and natural products have historically played a significant role in the treatment of a wide range of human health conditions. In recent years, there has been renewed interest in their use as part of integrated cancer therapies (Kamble and Gacche, 2019). Cancer encompasses a group of diseases characterized by uncontrolled and undifferentiated cell proliferation, with the potential to invade surrounding tissues and metastasize distant organs. According to the World Health Organization (WHO), cancer-related mortality is projected to rise, with an estimated 13.1 million deaths by the year 2030, as approximately 10 million new cancer cases are diagnosed annually (Boyle and Levin, 2008; Tai et al., 2020). The American Cancer Society (ACS) has expressed serious concern regarding the growing incidence and mortality associated with cancer (Parad, 2020). The highest cancer mortality rate was recorded in 1991. Since then, the death rate has steadily declined, resulting in a cumulative reduction of 29% by 2017— equivalent to an

estimated 2.9 million fewer cancer deaths than would have occurred had the 1991 peak rate persisted. This progress is largely attributed to sustained declines in mortality from the four most common cancers: lung, colorectal, breast, and prostate. Between 2008 and 2017, mortality rates from breast and colorectal cancers in women continued to decrease, while the rate for prostate cancer plateaued. Notably, lung cancer mortality decreased by approximately 3% annually from 2008 to 2013, culminating in the largest single-year decline in overall cancer mortality of 2.2% between 2016 and 2017. Despite these advancements, lung cancer remained the leading cause of cancer-related death in 2017, claiming more lives than brain, colorectal, breast, and prostate cancers combined (Siegel et al., 2020).

Cancer is influenced by a variety of risk factors, including environmental, genetic, and lifestyle-related elements, with age, smoking, and diet being among the most prominent contributors (Han et al., 2020). In the case of breast cancer, several interrelated factors have been implicated, such as sex, nutritional habits, alcohol consumption, environmental exposures, family history, lifestyle, and hormonal influences, both endogenous and exogenous (Abdulkareem, 2013). Globally, hundreds of thousands of women are diagnosed with breast cancer each year (Shareef et al., 2016), making it the second leading cause of cancer-related mortality among women (Azamjah et al., 2019). In many regions, particularly low-resource settings, the lack of access to routine mammographic screening often results in breast cancer being diagnosed at more advanced stages (Elsyana et al., 2016).

Among the various anticancer therapies, chemotherapy remains a primary systemic treatment that involves the administration of cytotoxic drugs to eliminate cancer cells (Shamsi and Islamian, 2017). However, the development of chemoresistance, a condition in which initially responsive tumor cells acquire resistance to specific anticancer agents, poses a major challenge to effective cancer treatment. This resistance often leads to therapeutic failure and disease progression. Many chemotherapeutic agents used in the treatment of breast cancer are derived from natural sources, including various parts of plants such as flowers, fruits, stems, and leaves, as well as from lichens and fungi. Given the limitations of conventional chemotherapy, particularly in terms of selectivity and systemic toxicity, there is a growing need for innovative drug delivery systems that enhance targeting specificity and therapeutic efficacy (Senapati et al., 2018). Natural products obtained from medicinal plants offer significant advantages due to their lower toxicity and reduced side effects compared to synthetic drugs, making them promising candidates for the development of safer anticancer therapies (Desai et al., 2008).

Recent studies have increasingly demonstrated the potential of various medicinal plants in the prevention and treatment of cancer. Many of these plants are rich in antioxidant compounds that have been shown to selectively target breast cancer cells without exerting toxic effects on normal, healthy cells. Antioxidants play a crucial role in neutralizing free radicals, thereby reducing oxidative stress, a known contributor to cancer development. Medicinal plants have long been valued for their therapeutic properties and are a significant source of bioactive compounds. Plant-derived products have offered promising avenues for cancer therapy and have contributed to greater public awareness regarding the importance of natural remedies in healthcare (Akindele et al., 2015). Phytochemicals, a diverse group of naturally occurring compounds in plants, provide a vast chemical space for drug discovery and development (Tai et al., 2020).

Plants also play a critical role in maintaining human health and nutrition. A wide range of plant species possess medicinal properties, and numerous pharmacologically active compounds have been identified in their extracts. However, many plant species with potential therapeutic value remain unexplored (Petrovska, 2012). Due to their bioactivity, medicinal plants offer a valuable platform for research in drug design and synthesis. For centuries, natural products have served as a foundation for therapeutic agents and biomedical innovation (Beutler, 2019). Their effectiveness is largely attributed to bioactive phytochemicals, which exhibit a variety of pharmacological mechanisms, including the activation of detoxifying enzymes such as glutathione S-transferase and the inhibition of cancer cell proliferation (Shareef et al., 2016; Chahardehi et al., 2021). These properties make medicinal plants a vital resource in the search for safer and more effective anticancer therapies.

In recent years, the use of herbal therapeutics and medicinal plant genera has gained widespread popularity in Pakistan for a variety of health-related purposes. These plants contain a diverse array of phytochemical constituents that function as secondary metabolites, including flavonoids, alkaloids, phenols, glycosides, steroids, and other bioactive compounds. Owing to their accessibility, cost-effectiveness, minimal side effects, and broad safety profiles, medicinal plants have become a major focus of ethnobotanical research. The therapeutic potential of plant-derived phytochemicals is increasingly recognized for their significant role in disease prevention and treatment. For instance, leptin has emerged as a novel target in breast cancer therapy. Quercetin, a well-studied flavonoid with chemopreventive potential found in numerous plant-based foods, has been shown to inhibit the proliferation of T47D

breast cancer cells by downregulating leptin gene expression and secretion (Elsyana et al., 2016). The anticancer properties of flavonoids are well-documented, and their global recognition highlights the importance of plants as a valuable resource for novel drug discovery (Idris et al., 2019).

Several natural products are currently utilized in anticancer therapies. *Cassia angustifolia* contains various bioactive compounds that exhibit promising anticancer activity (Ahmad et al., 2016). The present study was therefore designed to identify the bioactive constituents within the ethanolic for polar compounds and n-hexane for non-polar compounds from the crude extracts of Cassia angustifolia, and to evaluate their cytotoxic effects against the human breast cancer cell line MDA-MB-231.

MATERIALS AND METHODS

Extract preparation

In this experiment, Senna maki (*Cassia angustifolia*) dry leaves 50 g were collected to identify the bioactive compounds, and to evaluate the cytotoxic effect of crude extract on human cancer line MDA-MB-231. The finely grounded powder was subjected to 500 ml ethanolic and n-hexane (HPLC grade, Merck, Germany) solvents separately in flasks with the ratio 1:10 and were placed in the dark at 37℃ for three days with intermittent shaking (Bameri et al., 2013). The extract then evaporated in the rotary evaporator. The dry extract was stored at 4℃ for further utilization.

Phytochemical analysis

The qualitative analysis of phytochemical substances in crude extracts of *Cassia angustifolia.* To check the presence of secondary metabolites crude extract of *C. angustifolia* screened phytochemically. Standard protocols are used for determination of phenol contents, flavonoid and tannins.

Total phenolic contents of the ethanol and n-hexane extracts of *C. angustifolia* were determined by the Folin-Ciocalteu method. The standard protocols were followed (Islam et al., 2013). The chemicals were (0.5 ml) of Folin-Ciocalteu and sodium carbohydrate. Mixture was made into 1:10 ratio (extract and solvent) with 1ml of Folin reagent. Aqueous Na_2CO_3 added in solution. The absorbance was observed at 750 nm.

The total flavonoid contents of *C. angustifolia* extracts were determined by the aluminum chloride colorimetric method by Mohamed et al. (2013). Each plant extract (0.5ml) was prepared in (1:10 g/ml) ratio mixed with ethanol (1.5 ml). 0.1ml of aluminum chloride ($AlCl_3$) 10%, 1M of potassium acetate (0.1 ml) mixed with water and kept at room temperature for 30 minutes. Absorbance measured at 415 nm by using single beam of systronics UV visible spectrum. Calibration curves were prepared by quercetin solution at 12.5 concentrations.

Total tannin content was obtained by using FC reagent. Different concentrations of (0.1ml) extract along with distilled water were mixed with Folin-Denis reagent. 0.5% of N_2O_3 was added to it. Absorbance measured at 755 nm in 30 minutes. A different concentration of extract with solvents (Ethanol, and n-Hexane) along with water was mixed with 0.5 ml Folin Denis reagents (Negi et al., 2001). FC Reagent was Folin-Denis reagent.

Antioxidant analysis

The antioxidant activities of the n-hexane and ethanolic extracts of C. angustifolia and ascorbic acid will be evaluated with the DPPH method as described by Mohamed et al. (2013). DPPH solution (0.004%) prepared in n-hexane and ethanolic. Different concentrations were made using plant extract. DPPH (0.5 ml) added in it and absorbance was taken after 10 minutes at 517 nm. Ascorbic acid used as a standard, dissolve in distilled water to make standard solution of different concentration (10mg/100ml) or (100µg/ml).

In reducing power assay, the aliquots of various concentrations of the standard and extract (n-hexane and ethanol) of 1 ml dissolve in 2.5 ml potassium Ferri-cyanide added in it. After the incubation of 20 minutes trichloro-acetic acid added in it and centrifuge at 3000 rpm. Supernatant solution mixed water will be mixed with 2.5 ml distilled water and $FeCl_3$ (Islam et al., 2013).

1.0 ml of various concentrations of plant extracts (n-hexane and ethanolic) were made along with phosphate buffer. And then H_2O_2 solution was added in it. Absorbance was taken at 230 nm. Phosphate buffer runs as blank solution (Gurav et al., 2007).

In-vitro Anticancer potential

The plant efficiency was determined by performing MTT colorimetric assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide]. Cells were seeded at a density of 1 × 10^4 cells/well in a 96 well plate. The data was recorded morphologically after fixing the cell at 70% ethanol. The samples were stained using 5% crystal violet solution and visualized in bright field microscopy. Cell Adhesion assay was used to estimate the antiproliferative activities of the extracts against human cancer cell line MDA-MB231 and allowed to attach with the bottom over-night. Different

concentrations (50, 100, 250, 500µl) were used for cell treatment and assay was performed by fixing and staining it with 0.1% crystal violet. Optical density (OD) was taken at 570 nm.

For anticancer activities of the extract were determined by the MTT [3-(4, 5- dimethylthiazol-2 yl)-2,5-diphenyltratrazolic] was prepared at 5 mg/mL in phosphate-buffered saline (PBS). From this, 20 µl of MTT solution was added to each well containing 200 µl of culture medium so that the final concentration was 0.5 mg/mL on MDA-MB-231 cells (Maqbool et al., 2019). Cells were cultured in DMEM media containing 10% FBS (fetal bovine serum) and antibiotic (I%). Cells were incubated in incubator (5% CO_2) at 37℃. Cells were passed every 2–3 days using 0.25% trypsin-EDTA (Maqbool et al., 2019). When the cells were well grown in plate, exposed to MTT (25-50µl).

After the incubation of two hours, MTT medium was removed carefully by not disrupting layer at bottom (blue color). In each well, Dimethyl Sulphoxide (DMSO) was added and incubated it for 10-15 minutes at 37℃. The OD was measured at 570 nm to calculate the percentage of 100% control cells activity, percentage of treated cells viability calculate accordingly (Maqbool et al., 2019).

Viable cells percentage = (Absorbance of treated cells/Absorbance of control cells) × 100

Statistical Analysis

All the experiments were carried out in triplicate. Graph Pad Prism was used for statistical and graphical evaluations. Two-way ANOVA was applied for group data analysis and T-test would be used for other data (Maqbool et al., 2019)

RESULTS

Gas Chromatography-Mass Spectrometry Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis depicted that 29 compounds were detected at position 13 (Figure 1). The formula, structure and molecular weight of identified compounds in GC-MS are presented in Table S1. The peaks of the compounds were identified on the basis of retention time. Decane peak is detected at 11.56 min. n-decane is its synonym. It has 142 molecular weights. 1-Butanol, 3-methyl-, acetate is detected at 13.18 minutes. I obtained acetate, monoacetate, diacetate phenols and their different compounds. Benzene and its derivative at different retention are detected. Vitamin E is also detected in GC-MS results. Some compounds are detected twice at different retention times and have the same characteristics (Figure 1).

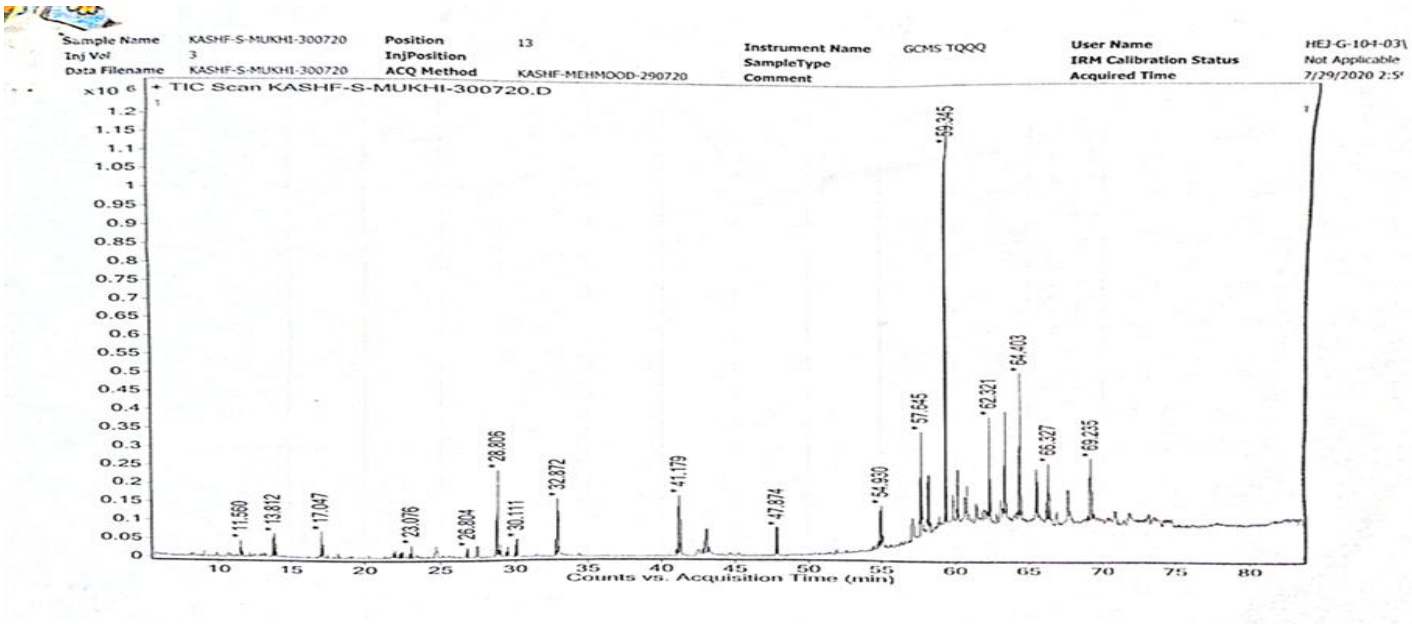


Figure 1. The highest peaks with retention time of various compounds detected in GC-MS report of senna Mukhi (cassia angustifolia).

Phytochemical analysis

Ethanol has higher extraction efficiency for phenolic and flavonoid compounds. The total phenolic content for ethanol was (±0.1318) noticeably higher than hexane's (±0.095), that showing ethanol has better ability to extract polar phenolic compounds (Figure 2A). Likewise, ethanol's total flavonoid content was ±0.196, which is substantially more than hexane's (±0.0131), indicating a significant difference in extracting flavonoids (Figure 2B). For tannins, however,

hexane recorded a slightly higher value (±0.3428), compared to ethanol (±0.3109), suggesting that despite being nonpolar, hexane can extract certain tannin components effectively (Figure 2C). Overall, such differences are largely affected by the polarity of the solvents, with ethanol favoring polar phytochemicals and hexane capturing some nonpolar tannin fractions.

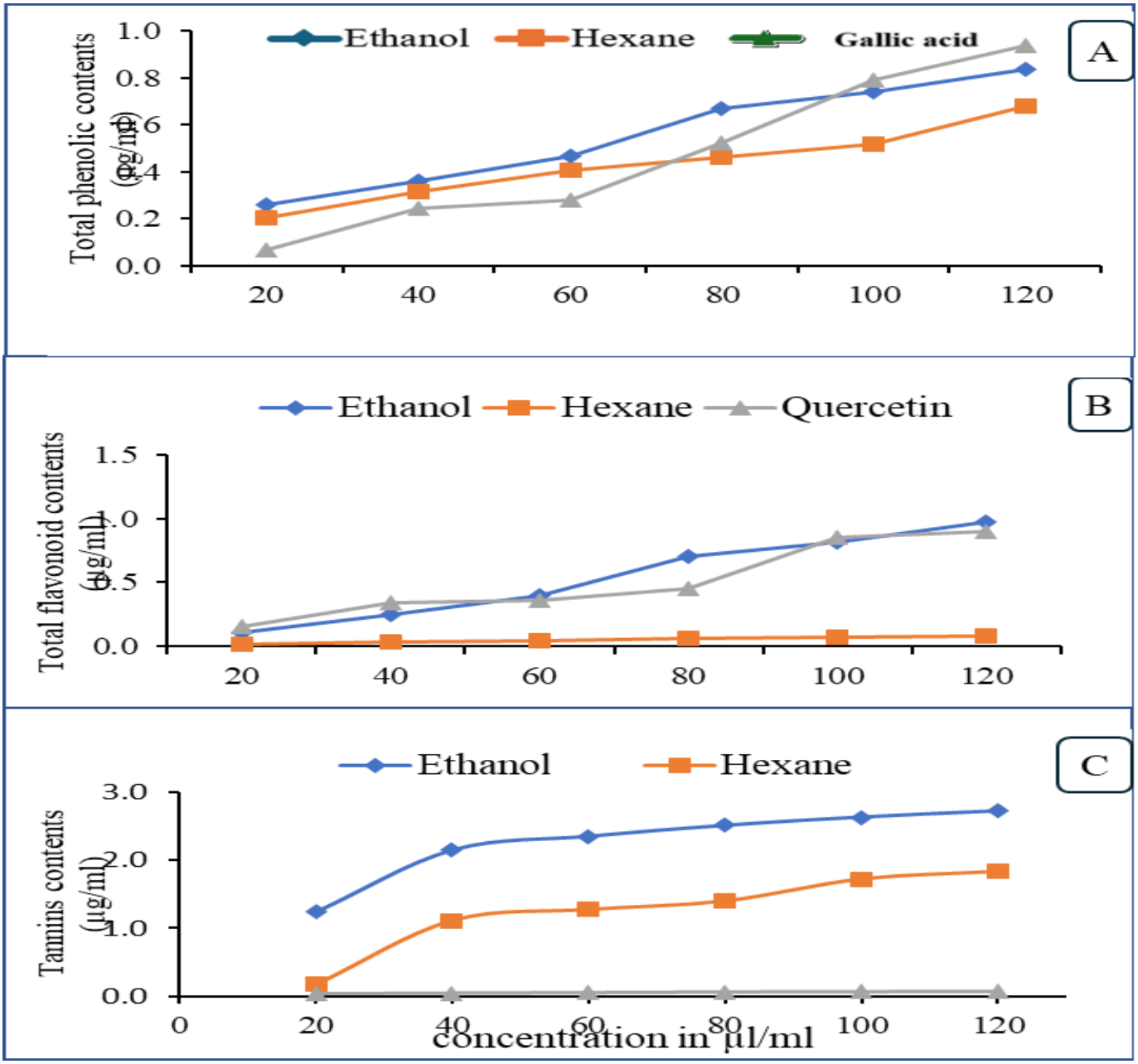


Figure 2. This graph shows the total phenol contents (A), total flavonoid contents (B), and total tannin contents (C) present in the plant extract of Cassia angustifolia. Concentrations are along the horizontal axis in µl/ml. Absorbance showing along the vertical axis. Blue line presenting ethanol contents, orange line presenting n-hexane contents and green line indicates the gallic acid contents, quercetin contents, and tannin acid

Antioxidant analysis

For DPPH activity, ethanol recorded a slightly higher value (±0.148) than hexane (±0.1396), showing a marginal advantage in neutralizing free radicals (Figure 3A). However, in the H_2O_2 scavenging test, hexane (±0.17205) outperformed ethanol (±0.0959), indicating greater effectiveness in quenching hydrogen peroxide radicals (Figure 3B). Similarly, in the reducing power assay, hexane achieved a higher reading (±0.0825) compared to ethanol (±0.0665), reflecting a stronger capacity for electron donation (Figure 3C). These results suggest that while ethanol extract is

more effective in DPPH scavenging, hexane extract shows better performance in hydrogen peroxide scavenging and reducing power.

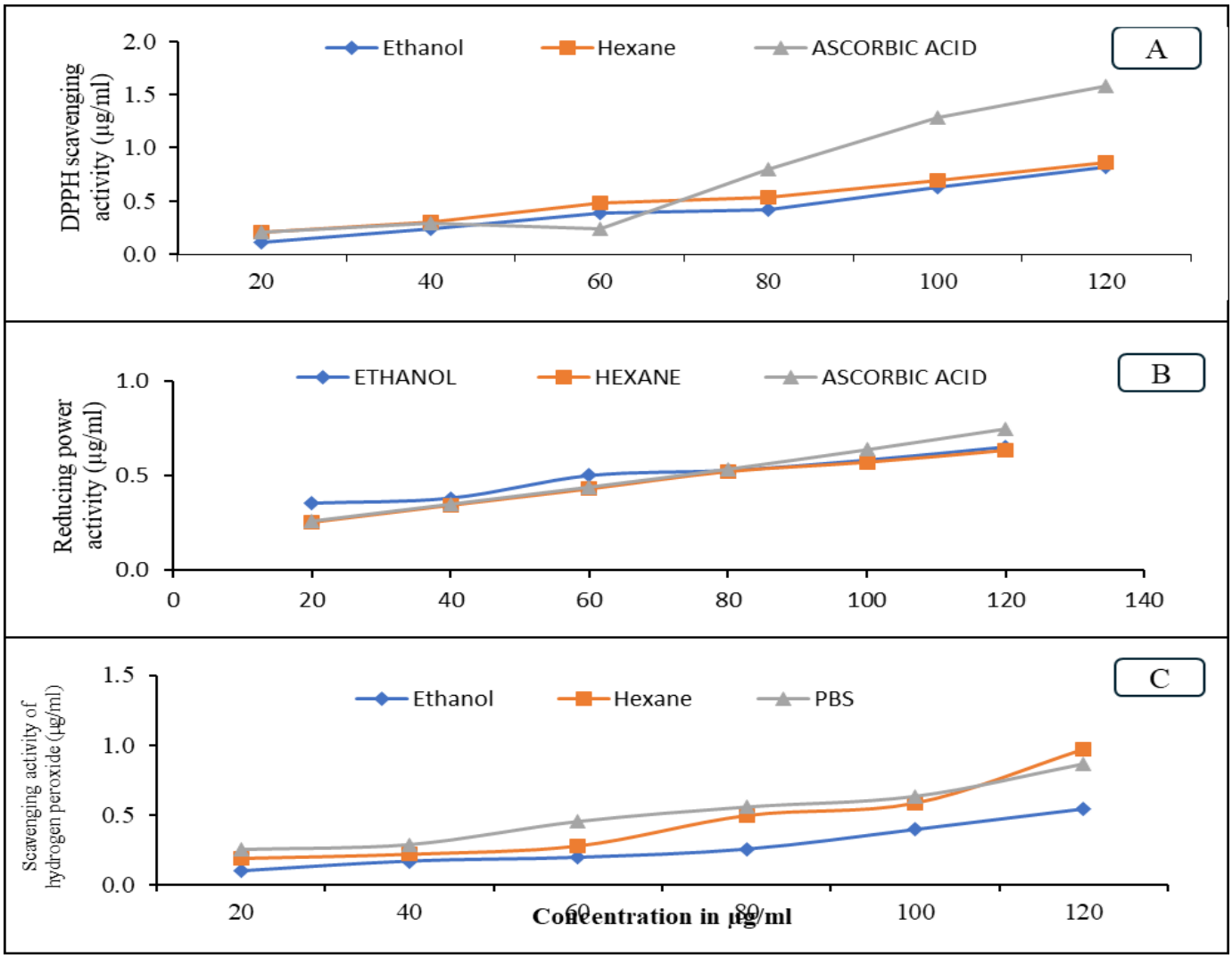


Figure 3: This graph shows the presence of DPPH free-radicals (A), reducing power of extract (B), and the scavenging activity of hydrogen peroxide (C) present in the plant extract of Cassia angustifolia. Concentrations are along the horizontal axis in µl/ml. Blue line presenting ethanol contents, orange line presenting n-hexane contents and green line indicates the ascorbic acid contents and phosphate buffer solution (PBS).

Extract morphological results

The morphological results of *Cassia angustifolia*'s ethanolic extract show various changes with different concentrations. Overall, as the concentration increases the number of cells decreases (Figure 4). Ethanolic extract's morphological observations were made, the samples treated with DMSO treated did not differ in cell morphology and the density as compared to the untreated cells. Furthermore, the differences in morphology and density of 50 µl/ml and 100 µl/ml. But as the concentration increases the difference becomes clearer, in case of 250 µl/ml majority of cells were round and others were elongated and not attached to the bottom of plate. In case of 500µl treatment most of the cells were dead leaving debris behind. A few cells are left which are round shapes (Figure 4).

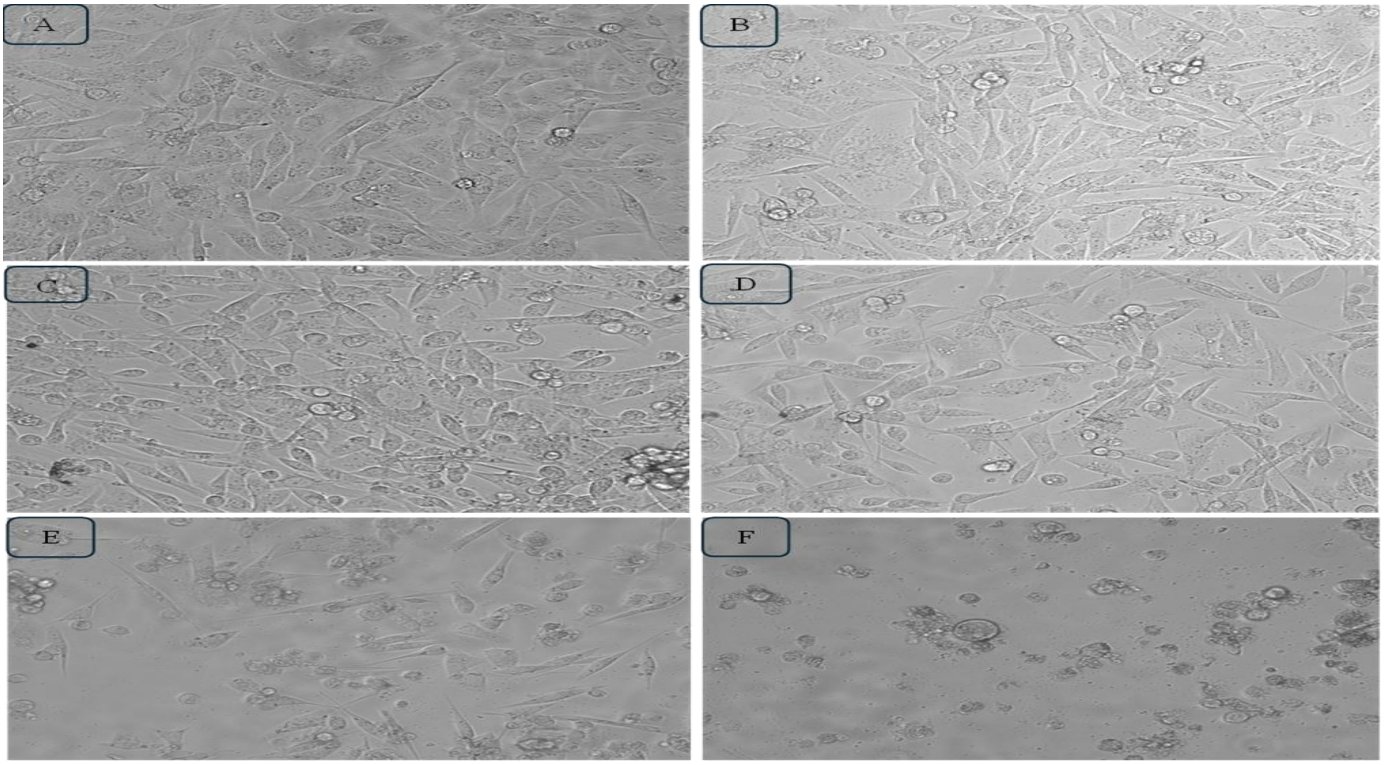


Figure 4. Morphological changes observed on MDA-MB231 cell line after in-vitro treatment with various concentrations of C. angustifolia ethanolic extracts (A) UT (B) DMSO (C) 50 µl/ml (D) 100 µl/ml (E) 250 µl/ml (F) 500 µl/ml

The morphological results of *Cassia angustifolia*'s n-hexane extract show various changes with application of different concentrations. The numbers of cells decreased as concentration increased (Figure 5). The samples treated with DMSO had no significant difference in cell morphology or cell density compared to the control or untreated cells. The 50 µl/ml concentration also had very negligible difference in the cell morphology as compared to the control well.

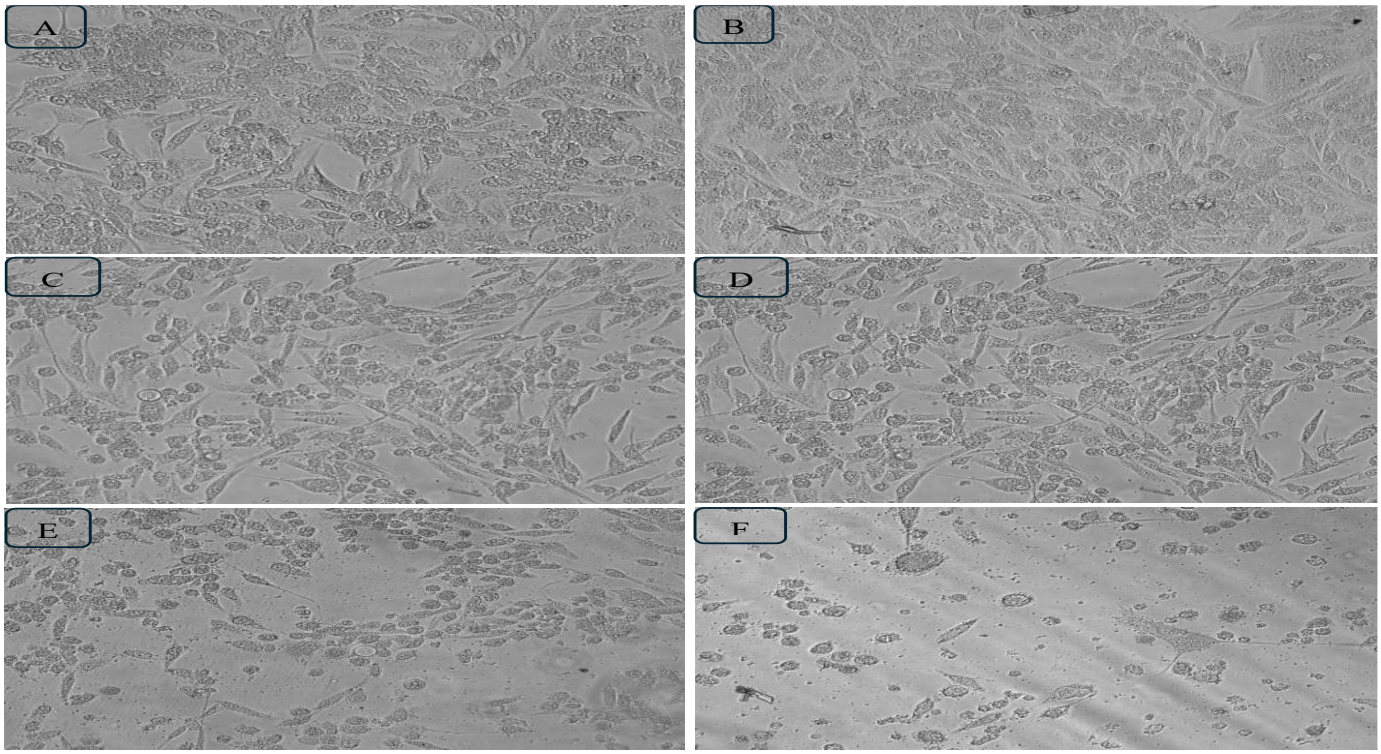


Figure 5. Morphological changes observed on MDA-MB231 cell line after in-vitro treatment with various concentrations of *C. angustifolia* n-hexane extracts (A) UT (B) DMSO (C) 50 µl/ml (D) 100 µl/ml (E) 250 µl/ml (F) 500 µl/ml

Cell Adhesion Assay

Figure 6A shows the data which were normalized with DMSO treated samples. From the present study, the ethanolic extract affected cell viability where increased concentration reduced cell viability. It was observed that in comparison to control samples the 50ul dosage had significant decrease in metabolic activity which was even lower for 100 µl/ml and 250 µl/ml concentration. The 500 µl/ml dose had the lowest metabolic activity. Figure 6B shows the data which was normalized with DMSO treated samples. The n-hexane extract affected the cell viability where the increase in concentration reduced cell viability. It was observed that compared to control samples 50 µl/ml and 100 µl/ml and 250 µl/ml had no significant decreases in cell viability. The 500 µl/ml dose had slightly lowest cell viability.

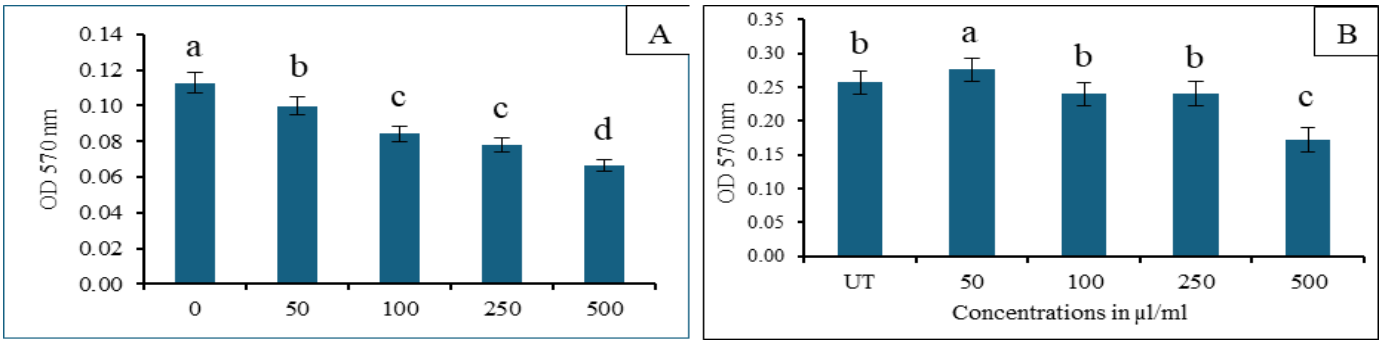


Figure 6. Graph shows cell viability of MDA-MB231 breast cancer cell line treated with ethanolic (A) and n-hexane (B) extract of *C. angustofolia*.

MTT assay

MTT assay was performed to check the viability of the cells. Results are analysed by graph using the GRAPH PAD PRISM (software).

The Figure 7A shows the data which was normalized with DMSO treated samples. According to data the ethanolic extract affected the metabolic activity in a dose dependent manner where increase in the concentration resulted in reduced metabolic activity. It was observed that compared to control samples the 50ul dosage had significant decrease in metabolic activity which was even lower for 100 µl/ml and 250 µl/ml concentration. The 500 µl/ml dose had the lowest metabolic activity.

Figure 7B shows that the treated samples were normalized by DMSO. Graph shows that the n-hexane extract effected the metabolic activity in a dose dependant manner where increase in concentration resulted in reduced metabolic activity. Moreover, when control samples compared with 50 µl/ml dosage showed slightly significant metabolic activity while 100 µl/ml had non-significant result. While 250 µl/ml had more effective result and 500 µl/ml had the lowest metabolic activity.

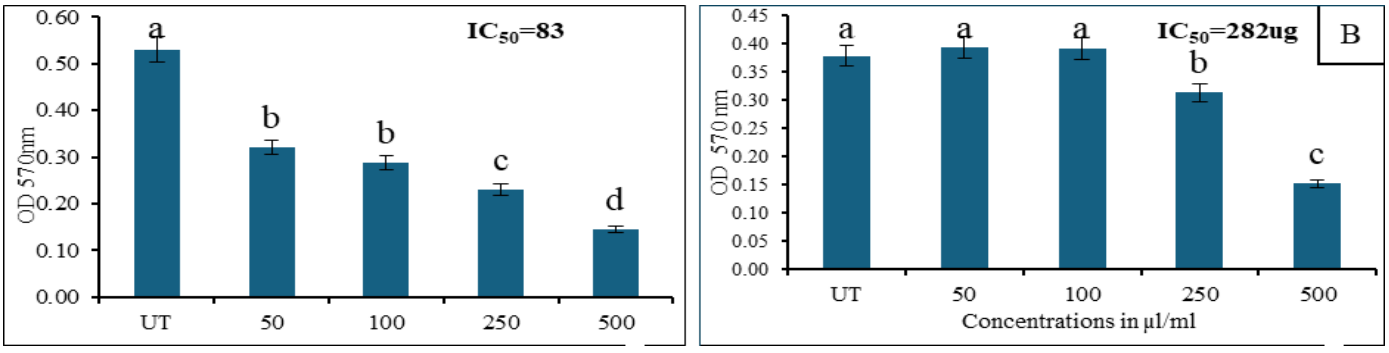


Figure 7. Graph shows the metabolic activity of MDA-MB231 breast cancer cells treated with ethanolic (A) and n-hexane (B) extract of *C. angustifolia*

Inhibition Concentration (IC_{50})

To calculate the cytotoxicity effect of two solvent ethanol and n-hexane of *C. angustifolia*' on breast cancer cell line MDA-MB231 was used. The values obtained from MTT were used to check the IC_{50} from online server https://www.aatbio.com/tools/ic50-calculator. IC_{50} of ethanolic extract was 83 and 283 of n-hexane extract of *C. angustifolia.*

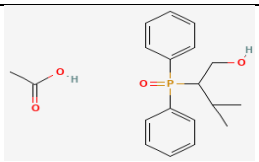
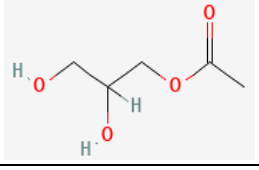
DISCUSSION

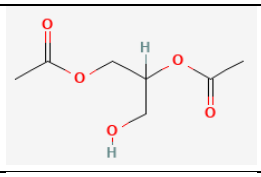
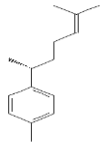
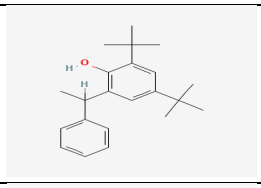
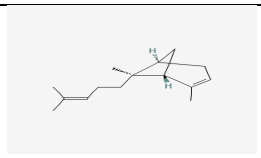
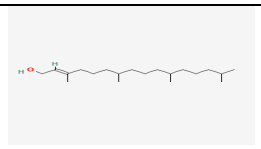
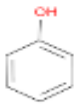
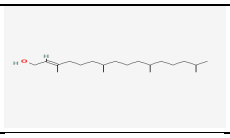
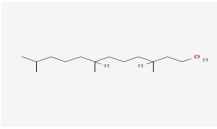
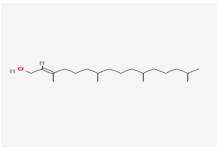
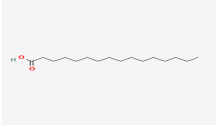
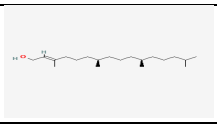
Phytochemical chemoprevention is a cost-effective and readily applied method to cancer control and management that is accepted. A mixture of phytochemicals, rather than a single agent, is thought responsible for the antitumor properties of plant-derived diets, rather than an individual agent (Kapinova et al., 2018). Analyzes have shown that Senna alexandrina leaf extract has alkaloids and flavonoids content of 0.483mg/g and 0.606mg/g, respectively (Leelavathi and Udayasri, 2018).

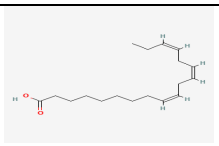
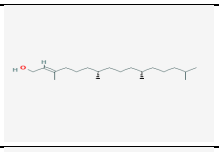
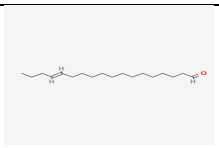
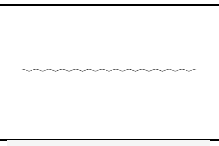
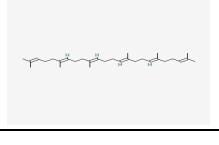
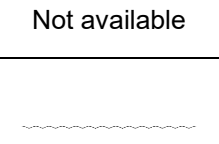
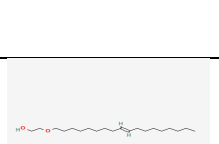
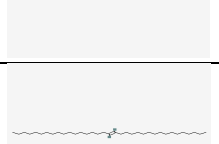
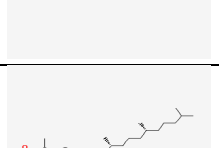
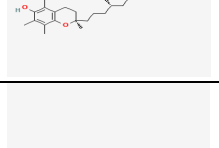
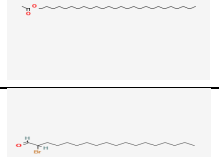
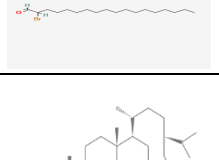
Gas chromatography–mass spectrometry (GC–MS) analysis of Cassia angustifolia revealed the presence of 29 phytochemical constituents. Comparative evaluation with existing literature identified squalene, monoacetate, diacetate, benzene derivatives, and phenolic compounds as major components, along with vitamin E, heptacosane, and several other bioactive metabolites (Parveen et al., 2016). Squalene, a hydrocarbon widely utilized in cosmetics and vaccine formulations, has been reported to exhibit cancer-preventive properties (Lozano-Grande et al., 2018). Phytol, an acyclic diterpene alcohol and a synthetic precursor of vitamin E, is commonly used in fragrances, household products, and detergents (Perumal et al., 2018). Phenolic compounds, characterized by hydroxyl functional groups, possess notable anticancer activity (Alagumuthu et al., 2018). Additionally, vitamin E, detected in the GC–MS profile, has been consistently documented as an anticancer agent (Abraham et al., 2019).

Morphological observations of plants extract breast cancer cells at different concentrations were observed in the study. The ethanolic extract's morphological observations were made, showing that the DMSO treated samples had no significant difference in cell morphology or cell density compared to the control or untreated cells. Ethanolic extract of *Cassia angustifolia* has more effective results as compared to the n-hexane. In the highest concentration 500µl treatment no cell is attached with the bottom of the plate, only debris leaving behind which indicates the death of the cell. The metabolic activity of the cells was determined on the breast cancer cell line MDA-MBA231. It is found that ethanolic extract of cassia angustifolia has more cell cytotoxicity as compared to the n-hexane extract. As shown in the current study (Table 1), the extract's secondary metabolites are known to be pharmacologically active ingredients with a wide range of therapeutic applications (Kamble and Gacche, 2019). They bear exact responsibility for a variety of actions, such as anticancer (Hossain and Nagooru, 2011). The phytoconstituents flavonoids and phenols have been widely documented to possess anticancer capabilities against a range of anticancer targets (Chahar et al., 2011; Iqbal et al., 2017), while some alkaloid has been reported to show antiproliferation effects against a variety of cancer types (Lu et al., 2012).

Table 1: The compounds with their formula, structure and molecular weight identified in Gas Chromatography-Mass Spectrometry (GC-MS) analysis

No. of peaks	Name of compound	Molecular weight	Formula	Retention time	Area sum%	Structures
1.	Decane	142	$C_{10}H_{22}$	11.56	2.29	
2.	Isoamyl acetate	130	$C_7H_{12}0_2$	13.812	4.62	
3.	Undecane	156	$C_{11}H_{24}$	13.958	1.85	
4.	Monoacetate	134	$C_5H_{10}O_4$	17.047	3.69	

5.	Diacetate	176	$C_7H_{12}O_5$	17.118	1.43	
6.	Benzene	202	$C_{51}H_{22}$	21.965	1.27	
7.	Phenol	206	$C_{14}H_{22}O$	22.367	0.87	
8.	Bicyclo [3.1.1]hept-2-ene	204	$C_{15}H_{24}$	22.474	0.88	
9.	2(4H)-Benzofuranone	180	$C_{11}H_{16}0_2$	23.076	2.39	
10.	Phenol	218	C15h220	26.804	2.04	
11.	3,7,11,15-Tetramethyl-2-hexadecane-1-0l	296	$C_{20}H_{40}O$	28.806	26.32	
12.	2-Hexadecane	280	$C_{20}H_{40}$	28.985	2.86	
13.	1-Dodecanol	228	$C_{15}H_{32}O$	28.992		
14.	3,7,11,15-Tetramethyl-2-hexadecane-1-ol	296	$C_{20}H_{40}O$	30.111	8.59	
15.	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	32.872	27.34	
16.	Phytol	296	$C_{18}H_{30}O_2$	42.179	44.38	

17.	9,12,15-Octadecatrienoic acid	278	$C_{18}H_{30}O_2$	43.053	23.11	
18.	3,7,11,15-Tetramethyl-2-hexadecane-1-ol	296	$C_{20}H_{40}O$	47.874	12.12	
19.	14-Octadecenal	266	$C_{18}H_{32}O$	54.93	16.37	
20.	Heptacosane	380	$C_{27}H_{56}$	57.645	17.39	
21.	Squalene	410	$C_{30}H_{50}$	59.345	100	
22.	2,6,10,14,18,22-Tetracosahexaene	410	$C_{30}H_{50}$	59.345		Not available
23.	Heptacosane	380	$C_{27}H_{56}$	60.144	11.81	
24.	Ethanol	312	$C_{20}H_{40}O_2$	62.321	32.17	
25.	17-Pentatriancontene	490	$C_{35}H_{70}$	63.394	38.21	
26.	Vitamin E	430	$C_{29}H_5O_2$	64.403	81.41	
27.	Hexacosanol	424	$C_{28}H_{56}O_2$	65.543	17.03	
28.	Octadecanal	346	$C_{18}H_{32}BrO$	66.327	25.44	
29.	Y-Sitosterol	414	$C_{29}H_{50}O$	69.235	38.12	

In a different investigation, Kamble and Gacche (2019) reported that the Sulforhodamine B (SRB) assay was used for the evaluation of the cytotoxic potential of meth-anolic extracts of Cassia occidentalis, Callistemon viminalis, Cleome viscosa, and Mimosa hamata against the human breast cancer cell line MCF-7. The investigation's findings showed that every one of the chosen plant extracts had a significant cytotoxic effect on MCF-7 cells, including *C. occidentalis* (IC50 = 70 µg/ml), *C. viminalis* (IC50 = 44 µg/ml), *C. viscosa* leaves (IC50 = 70 µg/ml), *C. viscosa* root (IC50 = 73.2 µg/ml), and *M. hamata* (IC50 = 65.8 µg/ml). By reducing the density of blood vessels, the plant extracts demonstrated strong antiangiogenic action in the CAM model. Out of all the samples that were examined, the C. viminalis extract showed the most antiangiogenic efficacy (67.76%).

The phytocompounds with anticancer potential were identified by GCMS employing n-hexane (HCA) and ethanol (ECA) solvent systems, according to Kalsoom et al. (2024). Cassia *angustifolia* extracts contained bioactive phytochemicals, according to GCMS analysis. With an IC50 value of 50 and 76 µg/mL, the extracts showed inhibition of tumor cells. Following treatment with CV extracts, there was a significant overexpression of the anti-apoptotic BCL-2 protein and a significant downregulation of the tumor suppressor TP53. The most potentially bio-active chemical towards the TP53 (-7.5) (kcal/mol) protein was found to be phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-, and isophytol (-6.9). The isolation and identification of three flavonoids, rutin, quercimeritrin and scutellarein, because of bioactivity-guided screening of *C. angustifolia* extracts, has also been reported. These flavonoids demonstrated significant anticancer activity against MCF-7 (IC50, 4.0 µg/µL), HeLa (IC50, 5.45 µg/µL), and Hep2 (IC50, 7.28 µg/µL), as well as low cytotoxicity against HCEC (IC50, 21.09 µg/µL). IC50 2.41 µg/mL demonstrated notable antioxidant activity against DPPH radical. Furthermore, *P. aeruginosa*, *E. cloacae*, *S. mercescens* and *S. typhi* microbiological growth may be inhibited by *C. angustifolia* extracts (Ahmad et al., 2016).

Ethanolic and n-hexane extracts of *Cassia angustifolia* demonstrated notable anticancer activity. Previous studies have indicated that secondary metabolites, particularly flavonoids, play a significant role in anticancer mechanisms (Piya and Anil, 2013). Therefore, the present study was designed to evaluate the anticancer potential of *C. angustifolia* extracts against the human breast cancer cell line MDA-MB-231. The findings support the hypothesis that flavonoids, previously identified in the methanolic and ethanolic extracts of *C. angustifolia* (Kalsoom et al., 2014), may be the key contributors to the observed activity. Comparable evidence exists in the literature, such as the flavonoid scutellarein isolated from *Scutellaria lateriflora*, which has been shown to induce apoptosis and significantly inhibit the proliferation of HT1080 human fibrosarcoma cells (Didem et al., 2010). Furthermore, in vivo studies have confirmed that scutellarein treatment leads to a marked reduction in tumor weight and size (Xiujuan et al., 2015). However, rutin has been shown to have anticancer properties by causing cell cycle inhibition and death in murine leukemia WEHI-3 cells (Lin et al., 2012). Additionally, the rutin possessed the ability to eradicate the MDA-MB-231 cell line's breast cancer cells (Aliye et al., 2014). The results of this investigation lend credence to the theory that *C. angustifolia* extracts' anticancer properties stem from the presence of secondary metabolites.

CONCLUSIONS

The present study demonstrated the anticancer potential of ethanolic and n-hexane extracts of *Cassia angustifolia* against the human breast cancer cell line MDA-MB-231 in vitro conditions. Both extracts exhibited significant inhibitory effects, suggesting that their bioactivity may be attributed to the presence of diverse secondary metabolites. These findings emphasize the broader importance of exploring medicinal plants as reservoirs of bioactive compounds with antioxidant, antibacterial, and anticancer properties, particularly in mitigating oxidative stress–induced degenerative disorders, various malignancies, and multidrug-resistant pathogens. Given its promising activity, *C. angustifolia* represents a potential candidate for further phytochemical characterization, mechanism-based investigations, and preclinical studies aimed at developing novel anticancer therapeutics.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

All the authors actively participated in finalizing the manuscript. All authors reviewed and approved the final version of the manuscript for publication.

COMPETING OF INTEREST

No potential conflict of interest was reported by the authors.

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