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# Phytochemical investigation and cytotoxic activity of hydro alcoholic fraction of *Trianthema decandra*

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The objective of our study was to perform phytochemical analysis and evaluate for cytotoxic activity of hydro alcoholic fraction (H1) of *Trianthema decandra* L. (Aizoaceae) against breast, liver and cervical cancers. Hydro alcoholic fraction was separated from methanolic extract, which was prepared by maceration method from aerial parts of *T. decandra* The GC-MS analysis confirms the presence of seventeen bioactive compounds which belongs to carbohydrates, terpenoids, alkaloids, cardiac glycosides, fatty acids and their esters or alcohols, and their presence were supported with Fourier Transform Infrared Spectroscopy (FT-IR) and preliminary phytochemical analysis. Most of the compounds are biologically active and are known to exhibit antimicrobial and cancer preventive properties. Hydro alcoholic fraction was subjected to morphological evaluation and MTT cell viability assay. It has exhibited significant cytotoxic activity and their $IC_{50}$ values were determined as 165.22 $\pm$ 1.53 µg/ml, 175.28 $\pm$ 1.7 µg/ml and 201.93 $\pm$ 1.33 µg/ml against MCF-7, HeLa and HepG2 cancer cells, respectively. This bioactive fraction has exhibited cytotoxicity relatively more against breast cancer than cervical and liver cancers.

**Keywords:** *Trianthema decandra*, HeLa, HepG2, MCF-7, MTT assay, GC-MS, cytotoxic.

## Introduction

Globally, cancer is the second leading cause of death and is responsible for an estimated 9.6 million deaths in 2018. According to incidence and mortality of cancers, breast cancer ranks second and fifth, liver cancer ranks sixth & fourth, cervical cancer ranks eighth & eighth respectively in the world scenario. Where as in India, breast cancer rank first in both studies, cervical cancer ranks third in incidence and fourth in mortality[1]. Cervical and breast cancers are two major cancers among Indian women. According to Globocan 2018, 6.6% of death occurs due to breast cancer, 8.2% of death occurs due to liver cancer and 3.2% of death occurs due to cervical cancer[2]. Cancer is uncontrolled growth of abnormal cells in the body. Many cancers arise from sites of infection, chronic irritation and inflammation. Dysregulated inflammation contributes too many diseases, including cancer[3]. Between 1981 and 2002, 48 out of 65 drugs approved for cancer treatment were natural products, based on natural products, or mimicked natural products in one form or another[4]. The National Cancer Institute (NCI) has screened approximately 35,000 plant species for potential anticancer activities[5]. Phytochemicals exhibit their pharmacological activities through various mechanisms but may fight against disease through suppression of the inflammatory response. Several cytotoxic, chemotherapeutic, and immunomodulating agents are available in allopathic medicine which are of high cost and are associated with serious side effects and morbidity to treat cancer. According to World Health Organization (WHO) approximately 70% of deaths from cancer occur in low- and middle-income countries. Still, the search continues for an ideal treatment that has effectiveness, minimal side effects and is cost-effective which is needed for economically challenged patients.

*Trianthema decandra* L. syn. *Zaleya decandra* (Aizoaceae) is commonly known as Gadabani (Hindi) and Tella galijeru[6] (Telugu). It is a prostrate, diffuse herb, widely found both in cultivated and waste lands of India, some parts of Asia, Australia, Africa and South America, abundantly grown in rainy season. Its leaves are edible. Traditionally used for the treatment of tooth ache, wound healing, anti-diabetic, fever, skin diseases and various parts used to treat the

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black quarter, bacterial infections, edema and rheumatitis[7]. Medicinal and pharmacological properties includes anti-inflammatory, analgesic, anti-diabetic, hepatoprotective, antidote to alcohol poisoning[8], antibacterial, antifungal, aphrodisiac, adaptogenic and antioxidant activities[9]. No scientific report is available on anticancer properties from this species till date. The objective of our study was to perform phytochemical analysis and screen for anticancer activity of hydro alcoholic fraction of *T. decandra* to evaluate its cytotoxic activity against breast, liver and cervical cancers.

## Materials and Methods

### Collection and Authentication of Plant Material

The aerial parts of *T. decandra* were collected in the monsoon season from surrounding places of Kakatiya University, Warangal, Telangana, India. The plants were authenticated by Dr. Ajmeera Raagan, Plants Systemic Laboratory, Department of Botany, Kakatiya University, Warangal, Telangana. A herbarium was deposited in the Department of Botany as Account no. KUW 1924 of Aizoaceae. The fresh aerial plant materials of *T. decandra* were carefully identified, collected, dried and then pulverized with mechanical grinder and sieved with no. 40 to get moderately coarse crude powder preserved in airtight container.

### Preparation of Extract and Fractions

The crude powder (1.21 kg) was macerated with methanol until extraction takes place exhaustively. Pooled up all the filtrates after quadruple maceration and the obtained methanolic extract was dried using rota evaporator and labeled as M1. The dried M1 extract is dissolved in ethanol and treated with petroleum ether, chloroform and water by successive solvent fractionation method, fractions were labeled as P1 (petroleum ether fraction), C1 (chloroform fraction) and H1 (Hydro alcoholic fraction). The percentage yield of M1, P1, C1, H1 are 5.97%, 0.68%, 1.76%, 2.68% respectively. The percentage yield was more with hydro alcoholic fraction than other fractions and subjected to phytochemical investigation and screening for anticancer properties by using *in vitro* bioassay methods.

### Phyto Chemical Analysis

The qualitative chemical tests carried out for the identification of the different phytoconstituents present in the hydro alcoholic fraction H1 by using standard methods[10-11]. Fourier transform infrared spectroscopy (FT-IR) analysis was performed using Shimadzu 8400S spectrophotometer. Gas chromatography- mass spectrometry (GC-MS) is a valuable tool for reliable detection of bioactive constituents. This fraction was analyzed by using a Shimadzu GCMS-QP2010 SE equipment with GC parameters (DB-624, fused silica capillary column ZB-5 (30 mm x 0.32 mm 1D X 0.25 μm), an electron ionization system (70 eV), helium as carrier gas at constant flow rate of 1.5 ml/min and an injection volume of 1 μl. Injector temperature 220°C; ion-source temperature 250°C with run time 30 min. Mass spectra with scan interval of 0.5 seconds and fragments from 350 to 650 m/z using turbo mass 5.2 with NIST ver 2.1 MS software data library.

### Cell Culture

Cell lines of HeLa, HepG2 and HEK-293 (ATCC) were sub-cultured in-house, in CDFD, Hyderabad. Cells were grown in Dulbecco's modified Eagles medium (Gibco Invitrogen, Paisley, UK) supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 1% penicillin (1000 U/mL), 1% streptomycin (1000 μg/mL) and 1% amphotericin (250 U/mL) and incubated with 5% $CO_2$ at 37°C. After 80% cell confluence, sub-cultures (conc. of 6 x $10^3$ cell/$cm^2$) were prepared with 0.25% (w/v) trypsin 0.53 mM EDTA solution and incubated at 37°C.

### Cytotoxic Activity

H1 fraction was qualitatively screened for cytotoxic activity by morphological evaluation by treated 12 hours post-seeding with 150 μg/ml and 300 μg/ml and normal HEK-293 cells with 300 μg/ml against HeLa, HepG2 and MCF-7 cancer cell lines and observed cells after 24 hrs, 48 hrs and 72 hrs. Images were aquired by Axiovert 200M phase contrast microscope (10X) using Axiovision Rel.4.2 software.

Quantitative estimation ($IC_{50}$ values) of cytotoxic activity of H1 fraction was determined by MTT cell viability assay, which is homogeneous colorimetric assay, based on the conversion of a tetrazolium salt MTT, a pale yellow substrate, to formazan, a purple dye. The intensity of the product color, measured at 550-620 nm, is directly proportional to the number of living cells in the culture. The cultured HeLa, MCF-7 and HepG2 cancer cells (100 μL per well) were added with H1 fraction concentrations ranging from 10 to 300 μg/ml (10, 50, 100, 150, 200, 250,

300 µg/ml) in triplicate after 24 hrs seeding and incubated for the 24 hrs, 48 hrs and 72 hrs period of time. To each well, 20 µL of culture medium, 15 µL MTT reagent per well which was made up in phosphate buffer saline (PBS) medium to a final concentration of 0.5 mg/mL. Cells were incubated for 3 hours at 37°C until intracellular purple formazan crystals are visible under microscope. The MTT reagent was removed and 100 µL of the dimethyl sulfoxide (DMSO) was added to each well and mixed gently on an orbital shaker for one hour at room temperature. Absorbance at OD 570 nm was measured for each well on an absorbance plate reader (Sunrise$^{TM}$, TECAN).

## Results

### Phytochemical Analysis

The preliminary qualitative phytochemical screening indicates the presence of alkaloids, carbohydrates, phenols or alcohols, terpenoids, cardiac glycosides, fatty acids, saponins, tannins and proteins. FT-IR spectrum was shown in Figure 1 and confirms the presence of phenols/alcohols, carboxylic acids, esters, aryl ketones, amines and amides functional groups which were given in Table 1.

### *GC-MS analysis*

From GC-MS analysis, phytochemical compounds were identified and the retention time, peak area, and molecular formula and their chemical nature and biological properties of compounds of their corresponding peaks were presented in Figure 2. The identified chemicals corresponding to the peaks were determined as follows: Mome inositol (26.57%), methyl ester of 2-hydroxy-valeric acid (18.9%), hexanoic acid, 2-methyl- (7.74%), imidazole, 2-amino-5-[(2-carboxy)vinyl]- (7.71%), decanoic acid (7.45%), allantoic acid (6.90%) are in major concentrations, dl-citrulline (3.8%), benz[e]azulene-3, 8-dione, 5-[(acetyloxy)methyl]-3a, 4, 6a, 7, 9, 10, 10a, 10b-octahydro-3a,10a-dihydroxy-2,10-dimethyl- (2.65%), octanoic acid (2.16%), 1,3-dioxolan-4-one, 5-benzyl-2-(1,1-dimethylethyl)-5-methyl- (1.37%), strogogenin (1.04%), 12, 15-octadecatrienoic acid, 2-[(trimethy lsilyl)oxy]-1-[[(trimethylsilyl) oxy]methyl] ethyl ester, (Z,Z,Z) (0.99%), 9n-hexadecanoic acid (0.89%), 1H-pyrazole, 4,5-dihydro-3-methyl- (0.66%), E-11-tetradecenol, trimethylsilyl ether (0.59%), dasycar pidan-1-methanol, acetate (ester) (CAS) (0.48%), malonic acid, bis (2-trimethylsily lethyl ester (0.41%) are in minimal concentrations. The biological properties which were mentioned in Table 2 are based on Dr. Duke's phytochemical and ethno botanical databases from Dr. Jim Duke of the Agricultural Research Service/USDA. The individual chemical compound name, fragmentation patterns of mass spectra and their structures of phytoconstituents identified in GC-MS spectrum were illustrated in Figure 3 (a-m).

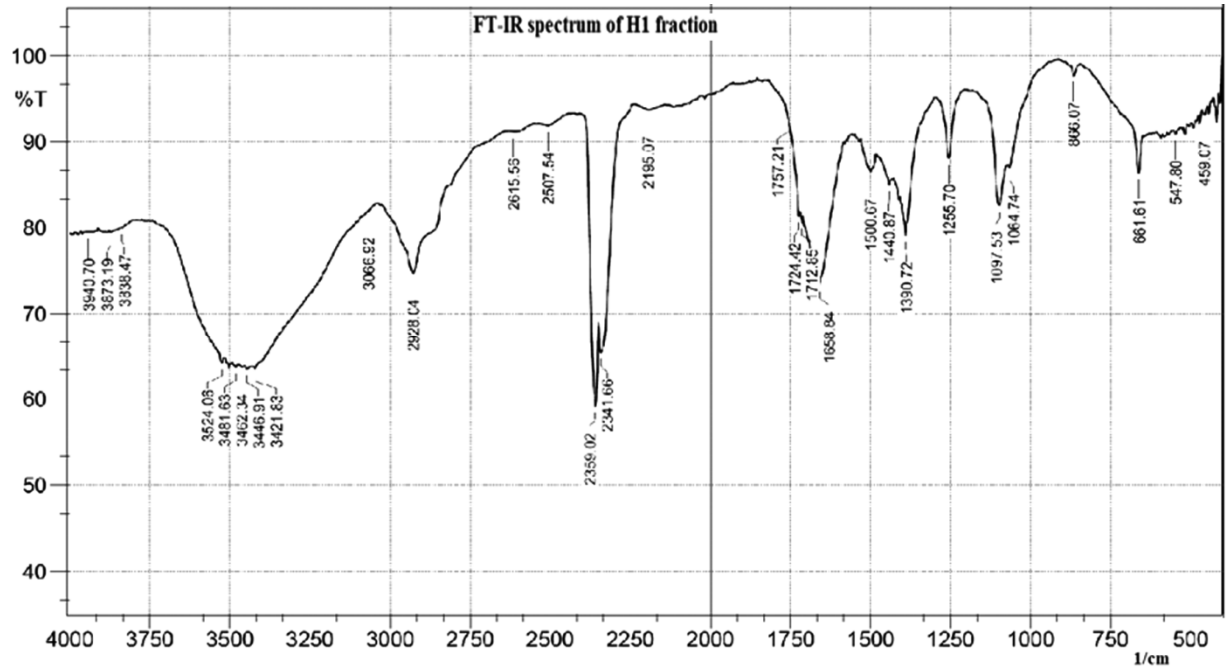


Fig. 1 — FT-IR spectrum of hydro alcoholic fraction (H1) of *Trianthema decandra.*

Table.1 — FT-IR spectroscopic profile of H1 fraction of *Trianthema decandra*

| Peak (Wave No. number cm-$^{1}$) | Intensity | Type of intensity | Bond | Type of vibration | Functional group assignment | Frequency (cm-$^{1}$) |
|---|---|---|---|---|---|---|
| 459, 547.8, | 93.09,90.9, | Medium | N-H | Bending | 1° amines | 900-660 |
| 866.07,661.61 | 86.3,97.66 | Weak | O-H | Bending (in plane) | Alcohols & phenols | 770-650 |
| 1064.7, 1097.53 | 87.0,82.6 | Strong | O-H | stretch | Alcohols | 1250-970 |
| | | Medium | $CH_3$ | bending (out of plane) | Alcohols & phenols | 1430-1330 |
| 1390.7 | 79.063 | | C-C-C | stretch | Alkanes & alkenes | 1470-1350 |
| 1255.7 | 88.066 | Medium | C-N | | Aryl ketones | 1390-1370 |
| | | | | | Amino | 1450-1100 |
| | | | | | | 1360-1080 |
| 1500.67 | 86.46 | Weak | C=C | Stretch | Arenes | 1500 |
| 1440.87 | 84.978 | | | bending | Ketones | 1450-1400 |
| 1658.84 | 73.33 | Strong | C=O | Stretch | Amides, carboxylic acids & derivatives | 1695-1630 |
| 1724.42 | 81.32 | | | | Aldehyde/ketones | 1740-1720 |
| 1712.85 | 80.59 | | | Stretch | Cyclobutanone | 1720-1710 |
| 1757.21 | 92.64 | | | | esters | 1750-1735 |
| 2341.6, 2359.02 | 59.17 | Medium | P-H | Stretch | Silane/ phosphorous gps/isocyanates | 2440-2280 |
| 2615.56 | 91.15 | Strong | O-H | Stretch | Carboxylic acids | 3300-2500 |
| 2928.04, 3066.9 | 74.73, 81.9 | Strong | C-H | Stretch | Alkanes | 3000-2850 |
| | | | | | Alkenes | 3100-3020 |
| 3421.8,3446.9, | 63.5, 63.4, | Variant | N-H | Stretch | Amines | 3500-3400 |
| 3462.3, 3481.6, | 63.6, 63.6, 64.1 | Weak | O-H (free) | Stretch | Phenols/alcohols | 3550-3200 |
| 3524.06 | | | | | =NOH oxime | 3600-3500 |
| 3873.1, 3838.4 | 79.4, 79.4 | Strong | C-H | Stretch | Aldehydes or ketones | 2690-3840 |

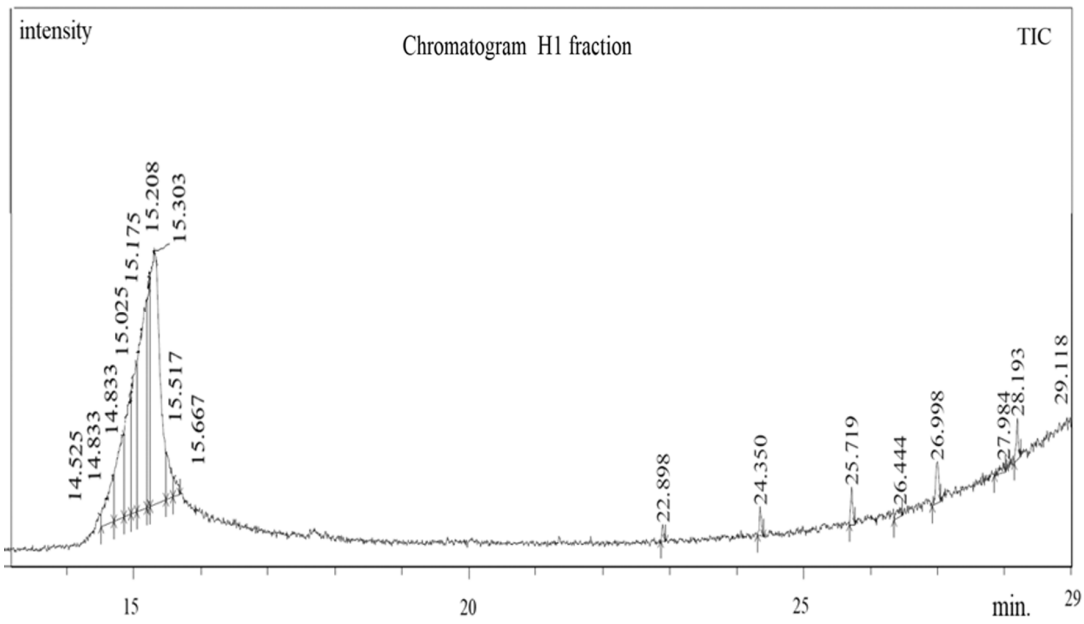


Fig. 2 — GC-MS chromatogram of hydro alcoholic fraction of *Trianthema decandra*.

**Cytotoxic Activity**

From the morphology studies it is concluded that H1 fraction has exhibited significant cytotoxicity at 300 µg/ml against HeLa, MCF-7 and HepG2 cancer cell lines. The morphological changes occurred in cancer cells after treatment with H1 fraction were

Table 2 — Phytochemical compounds identified in H1 fraction by GC-MS analysis.

| S. No | RT | Name of the compound | Mol. formula | Mol.wt | Peak area (%) | Nature of the compound | **Bio activity reported |
|---|---|---|---|---|---|---|---|
| 1 | 14.525 | dl-citrulline | $C_6H_{13}N_3O_3$ | 175 | 3.8 | Non essential amino acid | Stimulate the immune system and help detoxify ammonia [12] |
| 2 | 14.833 | Allantoic acid | $C_4H_8N_4O_4$ | 176 | 6.90 | Organic acid | Anti-cancer [13] |
| 3 | 14.833 | Imidazole, 2-amino-5-[(2-carboxy)vinyl]- | $C_6H_7N_3O_2$ | 153 | 7.71 | Imidazole nucleus | Antimicrobial, anti-cancer |
| 4 | 15.027 | Hexanoic acid, 2-methyl-(CAS) | $C_7H_{14}O2$ | 130 | 7.74 | Methyl caproic acid | Anti-cancer, flavour and fragrance agents |
| 5 | 15.175 | Methyl ester of 2-hydroxy-valeric acid | $C_6H_{12}O_3$ | 132 | 18.19 | Dicarboxylic acid ester | Not reported |
| 6 | 15.208 | Decanoic acid | $C_{10}H_{20}O_2$ | 172 | 7.45 | Capric acid | Anti-cancer, anti-convulsant |
| 7 | 15.303 | Mome inositol | $C_7H_{14}O_6$ | 194 | 26.57 | Sugar alcohol | Anti alopecic, anti cirrhotic, anti neuropathic, anti-cancer, cholesterolytic, lipotropic, Sweetener. |
| 8 | 15.517 | Octanoic Acid | $C_8H_{16}O_2$ | 144 | 2.16 | Caprylic acid | Anti-cancer, antimicrobial, weight reducing agent. |
| 9 | 15.667 | n-hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 0.89 | Fatty acid | Anti-cancer, Antioxidant, pesticide, flavor, 5-alpha reductase-inhibitor, Antifibrinolytic, nematicide, antialopecic |
| 10 | 22.898 | Malonic acid, bis(2-trimethylsilylethyl ester | $C_{13}H_{28}O_4Si_2$ | 304 | 0.41 | Malonate diester | Surgical adhesive |
| 11 | 24.35 | 1H-pyrazole, 4,5-dihydro-3-methyl- | $C_4H_8N_2$ | 84 | 0.66 | Methyl pyrazole | Not reported |
| 12 | 25.71 | 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[(trimethyl silyl)oxy] methyl] ethyl ester, (Z,Z,Z)- | $C_{27}H_{52}O_4Si_2$ | 496 | 0.99 | Linolenic acid | Cancer preventive, anti inflammatory, hypocholesterolemic, hepatoprotective, nematicide. |
| 13 | 26.44 | E-11-tetradecenol,trimethylsilyl ether | $C_{17}H_{36}OSi$ | 284 | 0.59 | Fatty alcoholic ether | Antimicrobial activity |
| 14 | 26.998 | 1,3-dioxolan-4-one, 5-benzyl-2-(1,1-dimethylethyl)-5-methyl | $C_{15}H_{20}O_3$ | 248 | 1.37 | Aromatic compound | Not reported |
| 15 | 27.984 | Dasycarpidan-1-methanol, acetate (ester) (CAS) | $C_{20}H_{26}N_2O_2$ | 326 | 0.48 | New aromatic compound | Not reported |
| 16 | 28.193 | Benz[e]azulene-3,8-dione, 5-[(acetyloxy)methyl]-3a,4,6a,7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-2,10-dimethyl- | $C_{19}H_{24}O_6$ | 348 | 2.65 | New aromatic compound | Not reported |
| 17 | 29.118 | Strogogenin | $C_{23}H_{30}O_8$ | 434 | 1.04 | Steroidal aglycone | Cardenolide, cytotoxic activity[13] |

**Activity source: Dr. Duke's phytochemical and ethno botanical databases.

compared with control and negative control which were illustrated in Figure 4. The elongated structured viable cells are morphologically turned into spherical shaped death cells after inhibition effect by test sample. In MCF-7 cells, the inhibition of activity was observed in wells at 150 µg/ml treatment after 72 hrs and death cells were present in wells at 300 µg/ml after 24 hrs, 48 hrs and 72 hrs. In HeLa & HepG2 cells, morphological changes observed at 300 µg/ml treatment after 24 hrs and death cells were present

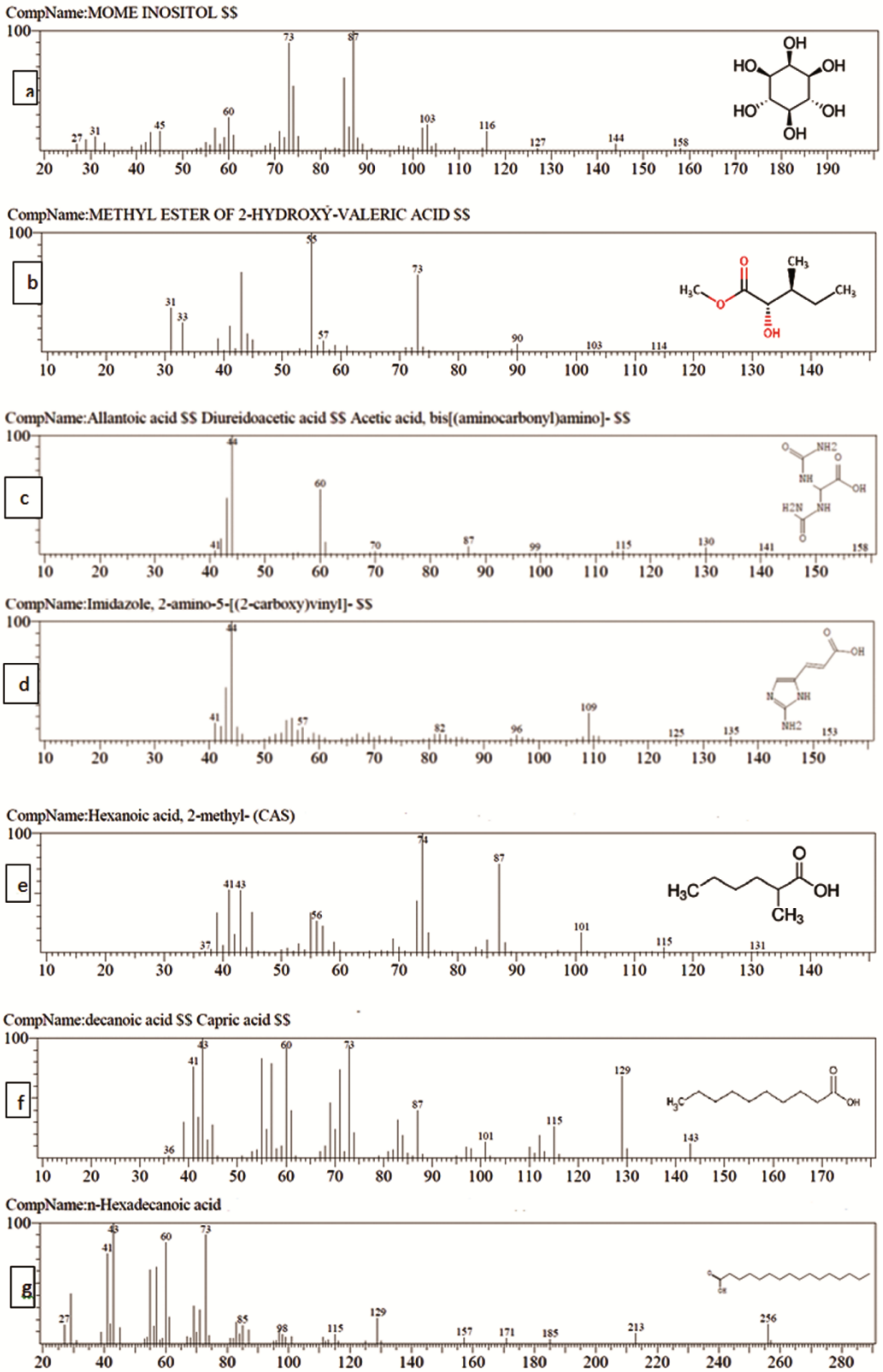


Fig. 3 — (a-g) Mass chromatograms and their structures of phyto components present in hydro alcoholic fraction H1. (*contd.*)

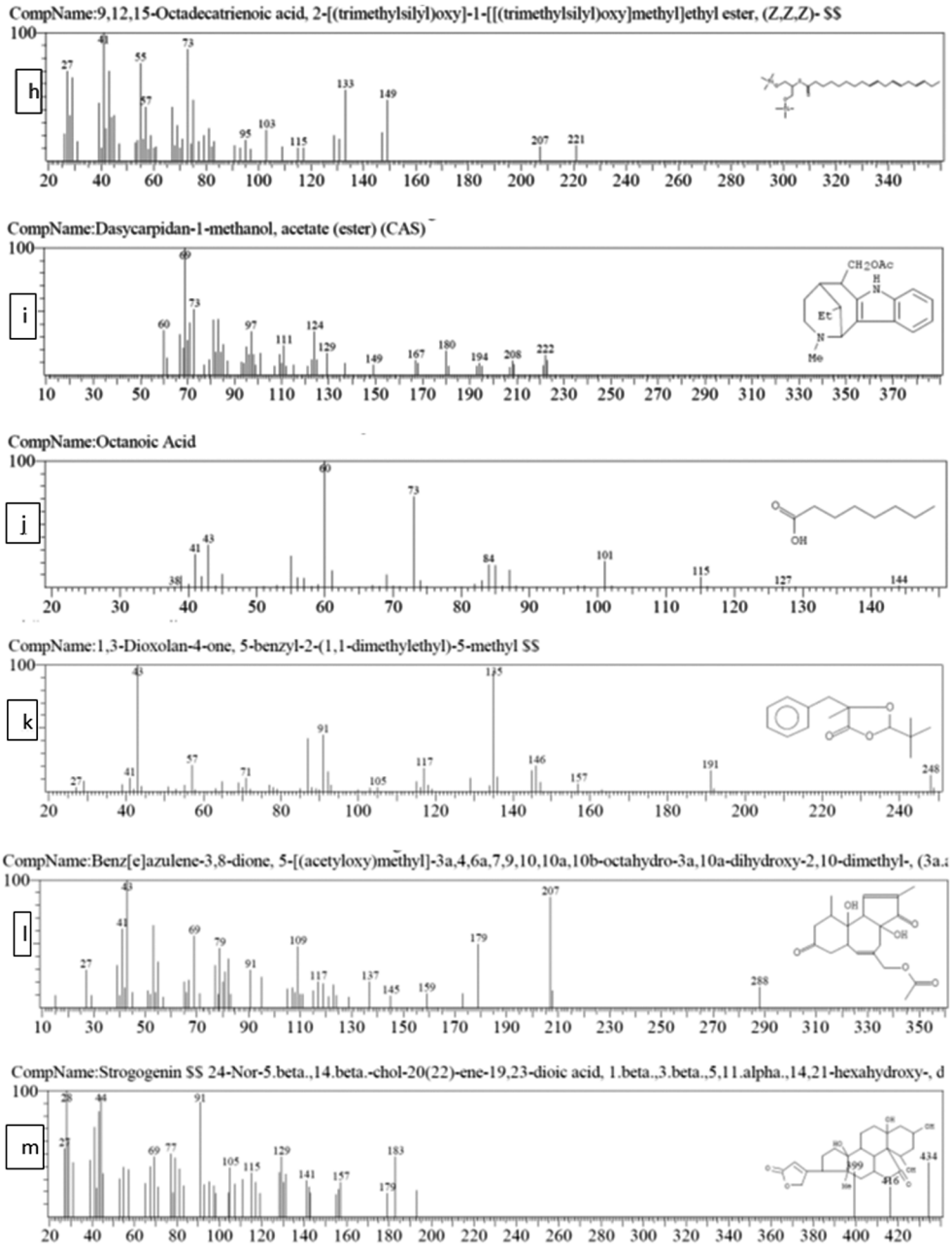


Fig. 3 — (h-m) Mass chromatograms and their structures of phyto components present in hydro alcoholic fraction H1.

after 48 and 72 hrs. There is no cytotoxic activity was observed after treatment of H1 at 150 µg/ml against HeLa and HepG2 cancer cells.

Treatment of H1 at 300 µg/ml against HEK-293 normal cells showed no significant morphological changes at any time point from 24 to 72 hrs when compared with control cells which were shown in Figure 5. The $IC_{50}$ values of H1 fraction against three cell lines at different timings were compared with values of standard drug doxorubicin and the values were given in Table 3. The cytotoxic effect was exhibited after 24 hrs and maximum effect observed after 72 hrs of treatment i.e $IC_{50}$ values against HeLa, HepG2 and MCF-7 cell lines were determined as 175.28 $\pm$ 1.7 µg/ml, 201.93 $\pm$ 1.33 µg/ml and 165.22 $\pm$ 1.53, µg/ml respectively. The percentage of viability cells observed at different concentrations of test fraction H1 against three cancer cells at 12 hrs, 48 hrs, 72 hrs timings were graphically represented in Figure 6. The cytotoxic activity ($IC_{50}$ values)

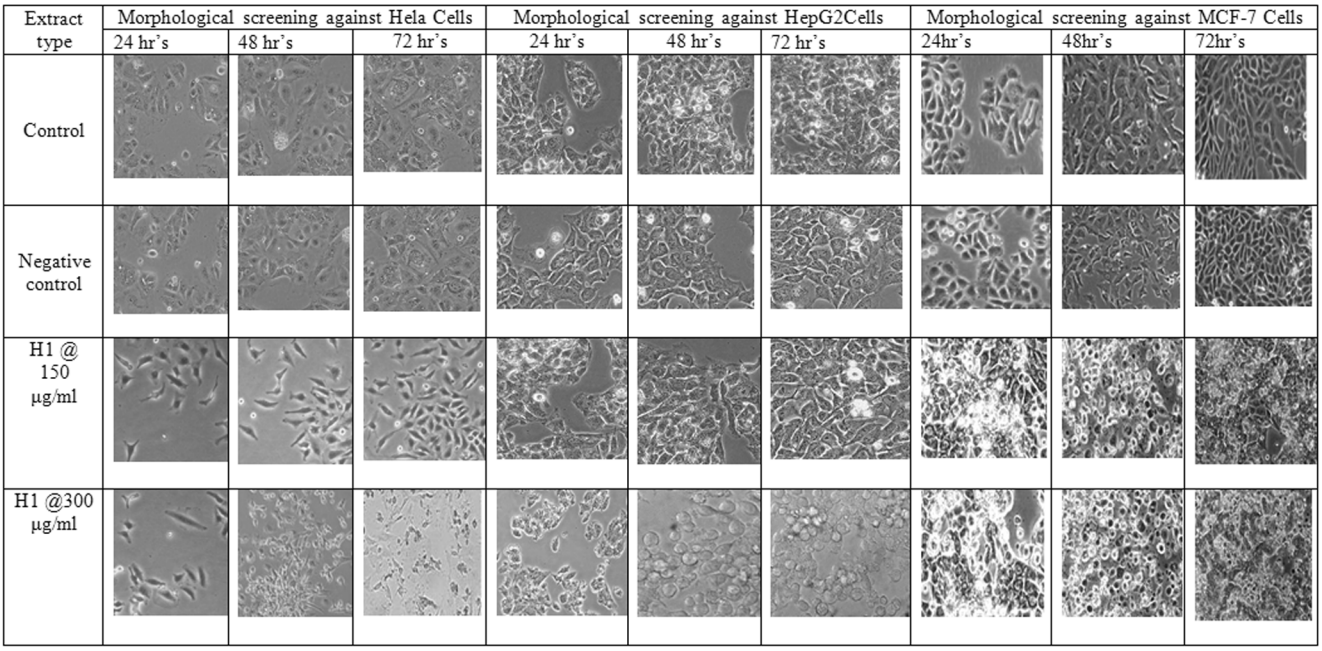


Fig. 4 — Morphological evaluation of H1 fraction against HeLa, HepG2 and MCF-7 cancer cell lines.

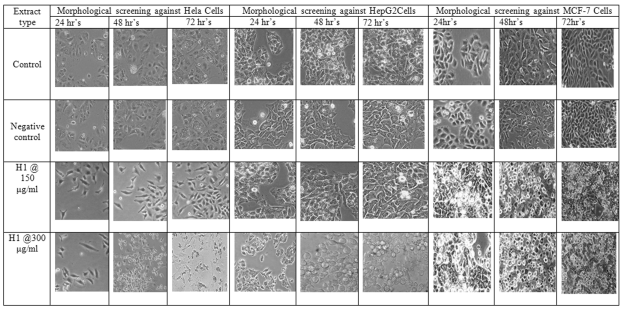


Fig. 5 — Morphological evaluation of hydroalcoholic fraction (H1) against HEK-293 normal cell lines.

exhibited by H1 fraction compared to $IC_{50}$ values of standard drug doxorubicin against various cancer cells were graphically represented in Figure 7.

## Discussion

GC-MS analysis revealed that seventeen phytoconstituents were present, among them the nature of compounds are known to be carbohydrate (mome inositol), amino acid (dl-citrulline), fatty acids and their alcohols or esters or ethers (hexanoic acid, 2-methyl-, decanoic acid, octanoic acid, n-hexadecanoic acid, 9, 12, 15-octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester,(Z, Z, Z)-, E-11-tetradecenol, trimethyl silyl ether), steroidal triterpenoid (strogogenin), esters of carboxylic acids (methyl ester of 2-hydroxy-valeric acid, malonic acid, bis(2-trimethylsilylethyl ester) moieties and remaining nitrogenous heterocyclic compounds (dasycarpidan-1-methanol, acetate, benz[e]azulene-3, 8-dione, 5-[(acetyloxy) methyl]-3a,4,6a, 7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-2,10-dim ethyl-, imidazole, 2-amino-5-[(2-carboxy)vinyl]-, 1H-pyrazole, 4,5-dihydro-3-methyl-) are expected to be alkaloids. Their confirmation was supported by the presence of peaks represents alcoholic (1250-950 $cm^{-1}$), carboxylic (1672.34 $cm^{-1}$), esters (1735.9, 1784.2 $cm^{-1}$), $1^0$, $2^0$, $3^0$ amines (1360-1080 $cm^{-1}$, 3500-3300 $cm^{-}$1 of C-N and N-H) as main functional groups from IR analysis and has shown positive results with corresponding chemical tests. From spectral analysis (FT-IR and GC-MS) it is clear that maximum concentration of alcoholic compounds and medium and long chain fatty acids are present in H1 fraction which is supported with information that, 49.365% of area is noted for the peak 3421.83 $cm^{-1}$ (O-H groups) and 29.767% of area is noted for the peak 2928.04 $cm^{-1}$ (alkanes/alkenes) in FT-IR spectrum which might be mome inositol (major compound) and fatty acids, remaining organic compounds.

Most of the identified compounds have been reported to possess interesting biological activities which were mentioned in Table 2. Mome inositol is carbocyclic sugar, the major component which is the precursor molecule of inositol hexaphosphate IP(6), appears to enhance the anticancer effect against breast metastases, and improve quality of life through

Table 3 — Cytotoxicity of H1 fraction against various cancers by MTT assay

| S.No | Type of cancer cell lines | Test sample | $IC_{50}$ values (µg/ml) | | |
|---|---|---|---|---|---|
| | | | 24 hrs | 48 hrs | 72 hrs |
| 1 | He La | H1 | 199.01 ± 1.27 | 186.13 ± 1.21 | 175.28 ± 1.7 |
| 2 | MCF-7 | H1 | 190.80 ± 0.85 | 175.54 ± 1.75 | 165.22 ± 1.53 |
| 3 | HeLa & MCF-7 | Doxorubicin | 52.37 ± 0.7 µM | 49.13 ± 0.5 µM | 48.62 ± 0.4 µM |
| 4 | HepG2 | H1 | 230.29 ± 1.31 | 210.71± 1.34 | 201.93 ± 1.33 |
| 5 | HepG2 | Doxorubicin | 65.57 ± 0.5µM | 61.37 ± 0.6 µM | 58.69 ± 0.9 µM |

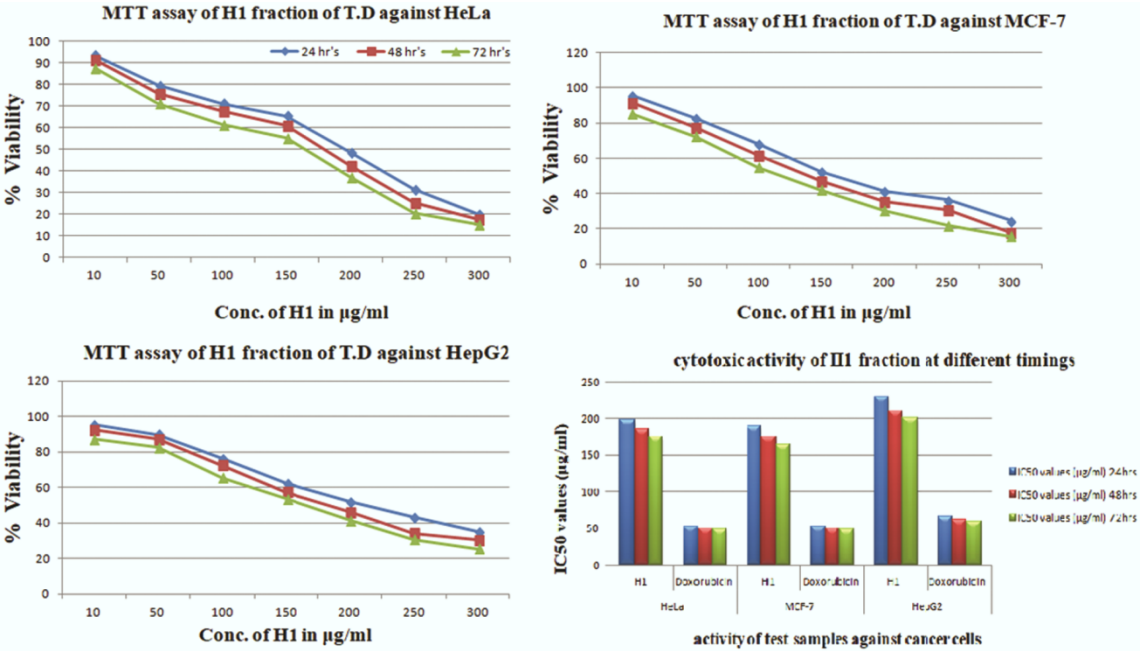


Fig. 6 — i), ii), iii) represents of MTT assay of H1 fraction of *Trianthema decandra* (T.D) after 24, 48 and 72 hrs treatment against HeLa, MCF-7 and HepG2 cancer cell lines and iv) Represents comparison of cytotoxic activity of H1 fraction against three cell lines along with standard drug doxorubicin at different timings.

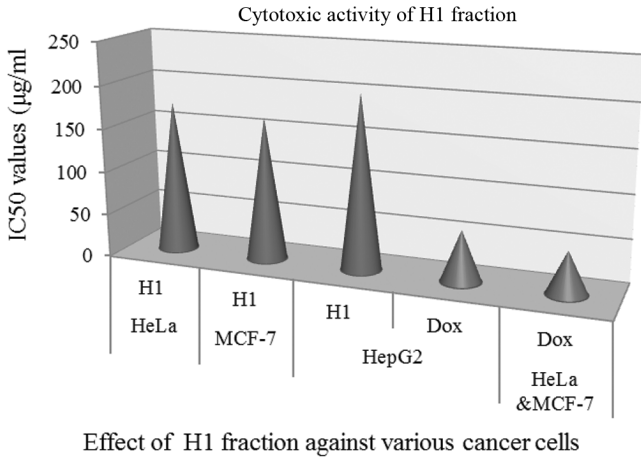


Fig. 7 — Graphical representation of cytotoxic activity of H1 against various cancer cell lines.

blocking progression along the cell cycle by counteracting the activation of the ERK pathway[14-16].

Alkaloids are natural compounds mostly contains basic nitrogen atoms and are produced by plants. Some alkaloids exhibits antitumor properties by regulates p21WAF1/CIP1 and induces apoptosis in p53-dependent (MCF-7) breast cancer cell line[17]. Dasycarpidan-1-methanol, acetate (ester) (CAS) is an ester of monoterpenoid indole alkaloid might be exhibited cytotoxic properties. Imidazole, 2-amino-5-[(2-carboxy) vinyl]- compound might have anticancer property against breast cancer because imidazole conjugates possess good PI3K inhibitory activity via PI3K/Akt/mTOR signaling pathway in breast cancer cell lines[18]. Many investigations demonstrated[19-20] that capric, caprylic and caproic acids are medium chain fatty acids exerted significant anticancer activity against breast and other cancer cells preferentially kill tumour cells without causing adverse effects on normal cells. Methyl hexanoic acid, decanoic acid,

octanoic acid are medium chain fatty acids, n-hexadecanoic acid is a long chain fatty acid and 9,12,15-octadecatrienoic acid, 2-[(trimethyl silyl) oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)- is an ester of poly unsaturated linolenic acid which are known to be anticancer compounds. α-linolenic acid and its metabolite docosahexaenoic acid (DHA) exhibits anticancer activity against breast cancer by which they could shift the pro-survival estrogen signal to a pro-apoptotic effect via GPER1-cAMP-PKA signaling response, blunting EGFR, Erk 1/2 and AKT activity in MCF-7 cells[21]. Most of the cardiac glycosides (CGs) are potential anticancer agents as they impart their antiproliferative effect by targeting multiple pathways. They exhibits cytotoxic activities against breast cancer via p-Akt signaling pathway was down regulated and p-ERK signaling pathway being activated in MCF-7 cells and have emerged[22]. Strogogenin is a cardenolide which is an aglycone, might be formed by lysis of glycosidic linkage of glycosides present in H1 fraction and might exhibits cytotoxic properties. The biological properties of the complex organic compounds ($14^{th}$, $15^{th}$, $16^{th}$ compounds mentioned in Table 2) are not reported but they might be possess cytotoxic properties or have exerted synergistic effect along with other compounds present in H1 fraction to exhibit significant cytotoxic activity.

From the results, H1 fraction has showed the significant cytotoxicity against breast, liver and cervical cancers which might be due to either synergistic effect of all the chemical components or a target specific molecular constituents by inhibiting the activation of receptor tyrosine kinases (RTKs) which influence ERK or other downstream signaling pathways, there by inhibition of cell growth, differentiation was observed. The higher cytotoxic effect is against breast cancer cells due to the presence of components which might be more likely to bind with cell specific functional proteins present in mitochondria of breast cancer cells than the cell specific biomolecules present in liver and cervical cancer cells and exerted maximum inhibition activity on breast cancer cells might be through lowering the over expression levels of the HER2 protein, either through gene amplification or through transcriptional deregulation via the PI3K/AKT/β-catenin downstream signaling pathway. Negative control results indicated that the solvent does not shown any effect against cancer cells and it also has shown non toxic activity on HEK-293 normal cell lines i.e the hydro alcoholic fraction of *T. decandra* has been proved to be safer for normal cell lines and effective in killing breast cancer cells.

From the above study it is concluded that, screening for anticancer properties of hydro alcoholic fraction H1 of *T. decandra* exhibited significant cytotoxic activity against breast cancer. The phytochemical investigation showed the existence of various compounds with different chemical structures. Further researches are needed for identification of bioactive compounds using various strategies to separate bioactive fractions or isolation and purification of individual constituents such as complex organic compounds and evaluate their cytotoxicity for both reported and non reported molecules and also elucidate the mechanism via cellular pathways of cytotoxic activities which may be used as templates for anticancer drug molecules.

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