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Research Article

Activities of Cinnamaldehyde from Boswellia Serrata on MCF-7 Breast Cancer Cell Line

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ABSTRACT

Boswellia serrata also known as Indian frankincense is known for its medical practice for thousands of years.B. serrata is recognized for its anti-proliferative and anti-inflammatory activities.Cinnamaldehyde is one of the common compounds derived from B. serrata from Methanolic extract (ME), Dichloromethanol extract (DME) and Hydrodistillate extracts. In this study, the effects of cinnamaldehyde on MCF-7 cell lines were investigated with hemolysis, cytotoxic effect, Antioxidant activity (Hydroxyl radical scavenging assay and Clonogenic Survival Assays. Cinnamaldehyde is a natural substance derived from B. serrata that plays an important role in the anti-proliferative activity. In this study, the anti-proliferative effect of cinnamaldehyde was investigated with its mechanisms of inhibition of proliferation growth at the morphological level. 100µM of ME was found to be more efficient to deliver the expected results while deriving less inhibition coefficient when compared with 100µM of DME. The study reveals the mechanisms of the anti-proliferative activities of cinnamaldehyde in MCF-7 breast cell lines, and further emphasizes that cinnamaldehyde could be a safe and effective natural agent to use in treating breast cancer. The study leads to the scope for further investigation of cinnamaldehyde in chemo therapy of breast cancer.

Keywords: Breast Cancer, MCF-7 cell line, Boswellia serrata, Cinnamaldehyde, Catechin.

INTRODUCTION

Cancer is the second major cause of death worldwide. Various chemotherapeutic drugs are established to treat and manage this devastating disease through systemic drug discovery and development[1]. According to the studies, Breast Cancer (BC) accounts for 22% and one of the most prevalent diseases worldwide[2]. Male breast cancer rates are less than 1 in one hundred thousand man-years [3]. BC hits the urban population three times greater than the rural populations [4].

Breast Cancer Treatment

Radiation therapy, chemotherapy therapy, hormonal therapy, and targeted therapy are the four major treatments to treat breast cancer. Chemotherapy targets to remove tumor or cancerous cells with the aid of medications. The present study is aimed to evaluate the anti-proliferative property of cinnamaldehyde of Boswellic acids from Boswellia serrata through Complementary Alternative Medicine (CAM) with the standard chemotherapeutic drug, Catechin on the most studied breast cancer cell lines, MCF-7. The experimental model, MCF-7 cell line, was derived from a breast adenocarcinomal tissue from a 69-year-old female. MCF-7 cell lines actively express the estrogen alpha along with progesterone, androgen, and glucocorticoid receptors hence it is helpful in understanding drug resistance which aids in development of chemotherapeutic drugs [5]. This research tries to address the usage of CAM as a supplement with the chemotherapeutic drug to minimize side effects. CAM includes herbal remedies, aromatherapy, biofeedback, massages, and so on. Here we have involved herbal or bioactive compound from B. serrata which has shown anticancerous property, previously [6].

Studies suggest that various bioactive compounds from B. serrata exhibit anti-proliferative properties and are known to initiate Apoptosis. According to Shashi Bhushan et al., (2007), an isomeric mixture of 3α, 24-dihydroxyurs-12-ene and 3α, 24-dihydroxyolean-12-ene of triterpenediol (TPD) from B. serrata has shown apoptosis (organized cell death) in tumor cells [7]. Various reports suggest that the resins derived from Boswellia species have medicinal properties. Researches on Boswellia have not been done vastly and the scientific data has many gaps which need to be filled to understand the prime importance of the species [8]. According to Sankpal UT et al., (2011), the gum resin derivative called acetyl-11-keto-beta-boswellic acid (AKBA) of B. serrata is found to be used in cancer therapeutics in traditional medicine. It also suggests that analog of boswellic acids have shown to inhibit the growth of cancer associated biomarkers along with the metastasis of human CRC in vivo conditions [9]. According to Lin HK et al., (2013) essential oils derived from Boswellia species have found to be biologically active. They suppressed the proliferation along with cytotoxicity of various cancer cell lines. Acetyl-11-keto-β-boswellic acid (AKBA) a purified boswellic acid has found to be effective to induce cytotoxic activities in vitro and vivo human models [10]. This study aims to study the activity of cinnamaldehyde extracted

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from resins of Boswellia serrata on MCF-7 breast cancer cell line.

Cinnamaldehyde is present in all the extracts in large percentage compared to the other terpenes. According to Lea-YeaChuang. et.al, 2012, Cinnamaldehyde caused inhibition of cellular mitogenesis, partly by promoting apoptosis in two human hepatoma cancer cell lines – HepG2 and Hep3B. Cinnamaldehyde blocked proliferation partly by promoting apoptosis in hepatoma cells. Therefore, Cinnamaldehyde may be useful as an anti-tumour agent [11].

Cinnamaldehyde can be used in various cancer chemotherapy including breast, colon and prostate cancers. Cinnamaldehyde and cinnamaldehyde derived compounds are candidates for the development of anticancer drugs that have received extensive research attention [12]. Hence the activity of it is evaluated on MCF-7 breast cancer cell line. MCF-7 is the most common model used to study breast cancer. The cell line is noninvasive by nature. It is widely utilized in the studies for its ability to express Estrogen receptor (ER) alpha in invasive human breast cancer [13]. MCF-7 has served as a fundamental reference cell line for many genomic studies, mostly because of the ability to generate an unlimited amount of RNA/DNA to enable validation and downstream functional studies [14].

METHODS AND MATERIALS

Essential extraction methods:

- Methanolic extract (ME) (Table I)
- Dichloromethanol extract (DME) (Table II)
- Hydrodistillate (Table III)

Chemical compositions of the extracted essential oils are determined using gas chromatography mass spectrometry (GC–MS). GCMS results (I, II and III respectively) are given in the table section. It was analyzed that Cinnamaldehyde is found to be common among all the three extracts.

GC-MS Analysis:

It was performed in Agilent Technologies connected with MS. Analytes were separated on a 30 m × 0.32 mm non-polar capillary column with a phase thickness of 1.0 µm and interfaced with a quadrupole mass spectrometer. The injector and interface temperature were kept at 270 °C and 320 °C respectively and the temperature was programmed from 60 °C to 260 °C at a rate of 2 °C /min. Helium was used as the carrier gas with a linear velocity of 74.6 cm/s and the total flow rate was 39.0 ml/min. The MS operating parameters were: ionization voltage 70 eV, scan rate 500 amu/sec.

Thin Layer Chromatography (TLC):

The samples were chromatographed with Thin Layer Chromatography (TLC) on silica gel 60F-TLC plates, developed using ternary-solvent system (hexane-chloroform-methanol, 5:5:0.5, v/v) and scanned at 260 nm [15].

MCF-7 Cell Culturing:

Cells were grown in EMEM (EBSS) media supplemented with 1% non-essential amino acids (NEAA), 10% foetal bovine serum (FBS) and 2mM glutamine. For our hormone-related studies, the medium is grown on low serum and phenol-red-free media was used [16].

CATALOGUE NO:[17]

Cell Line	Catalogue No.	Characteristics
MCF-7/S0.5	16022501	Human, Breast, Cancer, oestrogen receptor, MCF-7 Adapted to grow in low-serum media.

Docking Studies:

The molecular docking study is done to simulate the interaction between a tiny molecule and a protein which permits us to characterize the behavior of the binding site of target proteins. Docking efficiency can be increased by knowing the binding sites before docking process. If docking is done without any postulation of the binding site, it is known as blind docking [18]. Docking studies were carried out against Cinnamaldehyde and 5 different receptors to investigate and to access the binding efficiency of Cinnamaldehyde with 5 different anticancer receptors or proteins, a molecular docking imitation studies was undertaken. Estrogen receptors are the main receptor molecules for the breast cancer which mutates at various segments. Cinnamaldehyde acts upon various receptors but the below mentioned receptors were cautiously chosen on the basis of lately found mutation among the population. 5 protein ligands/receptors which were taken for docking studies are;

- 3ert: Human Estrogen Receptor Alpha Ligand-Binding Domain in Complex With 4hydroxytamoxifen
- 3hb5 Binary and Ternary Crystal Structures of a Novel Inhibitor Of 17 Beta-Hsd Type 1: A Lead Compound for Breast Cancer Therapy
- 3ols Crystal Structure of Estrogen Receptor Beta Ligand Binding Domain.
- 3s7s Crystal Structure of Human Placental Aromatase Complexed with Breast Cancer Drug Exemestane
- 5nqr Potent Inhibitors of Nudt5 Silence Hormone Signaling In Breast Cancer

Cytotoxic Assay:

The toxic effects of unknown compounds are determined invitro and performed by counting viable cells after staining with a vital dye. The activity of living cells is measured via mitochondrial dehydrogenases. This MTT method is simple, accurate and yields high reproducible results. The key component is (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, this is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using

acidified isopropanol or other solvents. The purple solution which is obtained is measured spectrophotometrically. The concomitant change in the amount of formazan formed is a result of increase or decrease in cell number, indicating the degree of cytotoxicity caused by the test compound [19].

Protocol:

104 – 106 cells were plated in 200 ml PBS in 96-well (flat bottom).

↓

20 ml of MTT solution is added and mixed well.

↓

Plate is incubated at 37˚C in dark for 4hours.

↓

An aliquot is removed for the analysis; 200 ml of acidic isopropanol is added and mixed well.

↓

The above solution is incubated for an hour at 37˚C in dark

↓

The plate is read in Elisa Reader at 570nm and measured OD (background wavelength is 630nm) [20]

Hemolysis Assay:

It is an assay used to determine the hemolytic effect of a compound. A suspension of red blood cells from a species specified by the customer

- The Negative control: PBS
- The Positive control: 1% SDS
- Test: DME and ME compound on MCF-7 cell lines.

Ten Separate test tubes containing MCF-7 cell lines were dosed with PBS, 1% SDS, DME and ME (4 different concentration) compounds respectively.

- Incubated in a water bath at 37°C with gentle agitation
- Each sample was centrifuged for released hemoglobin is quantified using a UV/Vis spectrometer at 405nm.
- Percent hemolysis is calculated at each sampling time point
- The percent hemolysis is calculated using the following formula:
  Percent Hemolysis = 100 x[(Absorbance of sample – Absorbance of negative control) ÷ (Absorbance of positive control – Absorbance of negative control)] [21]

Antioxidant (Hydroxyl Radical Scavenging) Assay:

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Various metabolic processes, UV radiations, smoke etc trigger the production of free radicals. Reactive oxygen species (ROS) includes superoxide anions (O2. -), hydroxyl radical (.OH), singlet oxygen, hydrogen peroxide (H2O2), ferric ion, nitric oxide (NO) etc. Excessive production of free radicals leads to Oxidative stress. The diseases associated with the ROS mainly depend on the balance of the pro-oxidant and the antioxidant concentration in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant. Free radicals have been implicated in causation of ailments such as cancer, inflammation, diabetes, liver cirrhosis, cardio vascular disease, Alzheimer's, Aging and acquired immunodeficiency syndrome. Reactive oxygen species (ROS) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid per oxidation. The increased production of toxic oxygen derivatives is considered a universal feature of stress conditions. Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecules and enzyme [22].

Clonogenic Assay:

It is an assay to test a given to analyze the reduction of the clonogenic survival of tumor cells. It is the method to determine cell reproductive death after treatment with ionizing radiation and effectiveness of other cytotoxic agents [23, 24].

Cancer cells were plated at $5x10^3$ cells/dish containing DMEM complete media and incubated at 37^0C, 5% CO_2 for 24 h. After 24 h, cells are treated with 50 and 100 g/ml of both *DME* and *ME* compound for 24 h. Cells are grown for 14 days and add the fresh media on fourth day. On 14^{th} day to produce colonies of >50 cells/ colony, remove the media from the dishes and washed with 1X PBS, cells were fixed with 3.7% formaldehyde for 5 min, followed by colonies were stained with 1 ml of 1% crystal violet in 1X PBS for 20-30 minutes on a platform.

Rinse the dishes three times with 1X PBS and air-dried, and then count the colonies. Finally take the picture at 4X by using inverted microscope).

RESULTS

Boswellic acids are terpenes found in small portions.

1. Compound has cinnamaldehyde which is active in the all the compounds and common in all the compounds according to the GCMS analysis. Table I, II & III depict the compounds present in Methanolic extract (ME) , Dichloromethanol extract (DME) & Hydrodistillate respectively.
2. Bioinformatics work: Breast Cancer (BC) denotes mutations. Estrogen receptors are the main receptor molecules for the breast cancer which mutates at various segments. Cinnamaldehyde is the common bio-active compound to be found in all the 5 receptors docking studies. So the compound cinnamaldehyde acts upon various receptors chosen in bioinformatics work. This docking study shows the mechanism of action of the cinnamaldehyde by how it binds to BC receptors.
3. Mutating enzymes are many; but 5 different proteins ligands were chosen which were commonly found among the population.

Table 1: Compounds Found In Sample 1 Of Methanolic Extract (Me)

Compound label	**Name**	**Hits**
Cpd 6: Acetophenone	Acetophenone	10
Cpd 12: Cinnamaldehyde, (E)-	**Cinnamaldehyde, (E)-**	**10**
Cpd 14: 2-Methoxy-4-vinylphenol	2-Methoxy-4-vinylphenol	10
Cpd 15: Cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl-	Cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl-	10
Cpd 17: Cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl-	Cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl-	6
Cpd 21: Vanillin	Vanillin	9
Cpd 27: Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	10
Cpd 29: Benzene, (1-methoxyethyl)-	Benzene, (1-methoxyethyl)-	8
Cpd 39: n-Pentadecanol	n-Pentadecanol	10

Table 2: Compound Found in sample 2 of DME

Compound label	**Name**	**Hits**
Cpd 1: Cyclohexene, 4- methylene-1-(1-methylethyl)	Cyclohexene, 4- methylene-1-(1-methylethyl)	10
Cpd 2: (+)-4-Carene	(+)-4-Carene	10
Cpd 3: endo-Borneol	: endo-Borneol	10
Cpd 4: 3-Cyclohexen-1-ol, 4- methyl-1-(1-methylethyl)-, (R)-	Cyclohexen-1-ol, 4- methyl-1-(1-methylethyl)-, (R)-	6
Cpd 5: L-.alpha.-Terpineol	L-.alpha.-Terpineol	10
Cpd 6: Cinnamaldehyde, (E)-	**Cinnamaldehyde, (E)-**	**10**
Cpd 7: 3,6-Dimethyl-2- nitrobenzaldehyde	3,6-Dimethyl-2- nitrobenzaldehyde	7
Cpd 9: Bicyclo[7.2.0]undec-4- ene, 4,11,11-trimethyl-8- methylene	Bicyclo[7.2.0]undec-4- ene, 4,11,11-trimethyl-8-methylene	10
Cpd 10: Bicyclo[3.1.1]hept-2- ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)	Bicyclo[3.1.1]hept-2- ene, 2,6-dimethyl-6-(4- methyl-3-pentenyl	10
Cpd 11: 2-Hydroxy-1,8-naphthyridine	2-Hydroxy-1,8-naphthyridine	10
Cpd 12: 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	10
Cpd 13: Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	10
Cpd 14: Benzoic acid, 2-(1-oxopropyl)-	Benzoic acid, 2-(1-oxopropyl)-	7
Cpd 15: (R)-(-)-14-Methyl-8-hexadecyn-1-ol	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	10
Cpd 16: N-Benzyloxy-2,2-bis(trifluoromethyl)aziridine	N-Benzyloxy-2,2-bis(trifluoromethyl)aziridine	8
Cpd 17: Tetradecanoic acid	Tetradecanoic acid	7
Cpd 22: 1-Nonylcycloheptane	1-Nonylcycloheptane	10
Cpd 24: Andrographolide	Andrographolide	10
Cpd 29: 1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl-, methyl ester,[1R-(1.alpha.,4a.beta.,4b.alpha.,7.alpha.,10a.alpha.)]-	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl-, methyl ester, [1R-(1.alpha.,4a.beta.,4b.alpha.,7.alpha.,10a.alpha.)]-	7
Cpd 36: Methyl abietate	Methyl abietate	7
Cpd 32: 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	8

Table 3: Compounds Found in Sample 3 Of Hydrodistillate

Compound label	Name	Hits
Cpd 13: N-Benzyloxy-2,2-bis(trifluoromethyl)aziridie	N-Benzyloxy-2,2-bis(trifluoromethyl)aziridie	10
Cpd 35: 4-Hydroxy-2-methylacetophenone	4-Hydroxy-2-methylacetophenone	10
Cpd 73: (S)-6,6-Dimethyl-2-azaspiro[4.4]non-1-ene	(S)-6,6-Dimethyl-2-azaspiro[4.4]non-1-ene	10
Cpd 74: Tricyclo[4.3.1.0(2,5)]decane	Tricyclo[4.3.1.0(2,5)]decane	10
Cpd 76: Cis-8-methyl-exo-tricyclo[5.2.1.0(2.6)]decane	Cis-8-methyl-exo-tricyclo[5.2.1.0(2.6)]decane	10

4. Structure of breast cancer receptors were retrieved from the Protein Data Bank and the structures of flavonoids compounds have been collected from PubChem databases. Molecular docking and drug likeness studies were performed for those natural compounds to evaluate and analyze the anti-proliferative breast cancer activity. Binding affinity is observed. Lower the scores, higher the binding affinity (BA). Accordingly, 3OLS Crystal Structure of Estrogen Receptor Beta Ligand Binding Domain has showing least binding affinity (-3) and hence is considered to be best among the 5 receptors. The results of this study can be implemented in the drug designing pipeline.
5. Catechin is a flavan-3-ol, a type of natural phenol and antioxidant. It is a plant secondary metabolite. It belongs to the group of flavan-3-ols (or simply flavanols), part of the chemical family of flavonoids. It has anticancer properties and it's widely used as chemical formulations hence Catechin is used as standard control in this study with our extracted test samples.
6. According to Cytotoxic assay (MTT Assay) Fig.2 & 3 and Table IV, it can be interpreted that 100µM has lesser IC50 value and is more potent than the 50 and 100 µM of DME.
7. From hemolysis studies (Table V, VI & Fig .4), we are able to conclude that 320 µM of DME and ME are equally potent. 100µg/mL of DME is efficient to exhibit its Antioxidant activity according to the Antioxidant assay/ Hydroxyl radical scavenging assay.
8. From Clonogenic Survival Assay (Fig 5 & 6), it is evident that the MCF-7 cells treated with 50 and 100 µM/ml of DME compound for 24 h showed around 50% of inhibition at 50 µM/ml and around 20 % inhibition of colonies forming ability at 100 µM/ml. However, the compound ME has shows inhibition of 15% at 50 µM/ml and 100% inhibition at 100 µM/ml of colony forming capability. The summation of the various assays result suggests that ME has more significant colony forming inhibition abilities compare to DME, which also shows inhibition at higher concentration.

Overall, these results show that both ME and DME have showed inhibition of the growth of MCF-7cells by preventing the formation of colony and thereby inducing apoptosis.

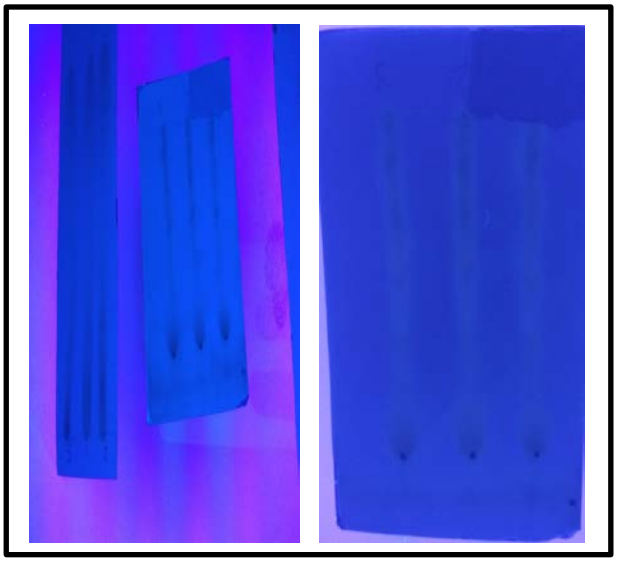

Fig. 1: Run 1 & 2: Thin Layer chromatography of Standard (Catechin) , DCM extract, Methanolic extract.

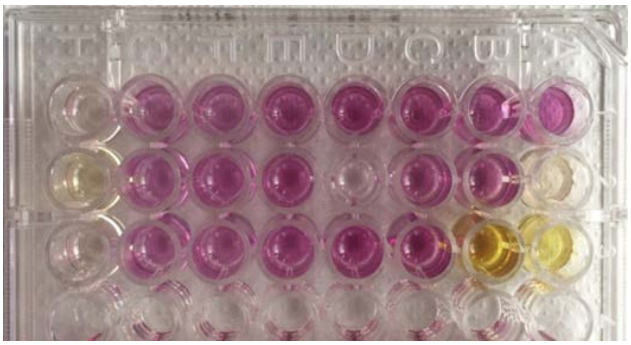

Fig. 2: Cytotoxic assay (MTT Assay; ELISA PLATE)

DISCUSSION

The present study assessed the psychological problems and its coping strategies of elderly persons residing in old age home. The study results reveal that majority 60% of them were had moderate level of psychological problems like

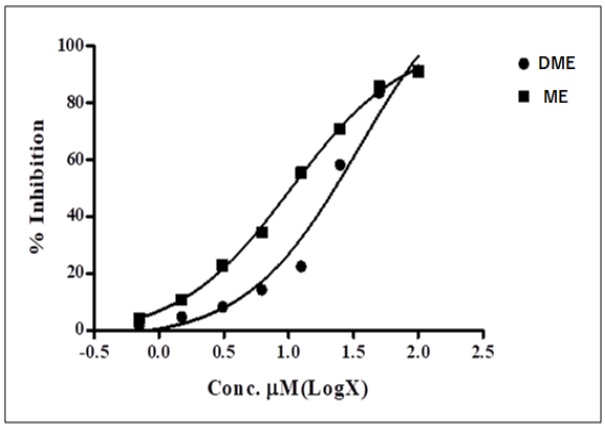

Fig. 3: Determination of Percent Inhibition (IC50) of DME and ME on MCF-7 cell lines

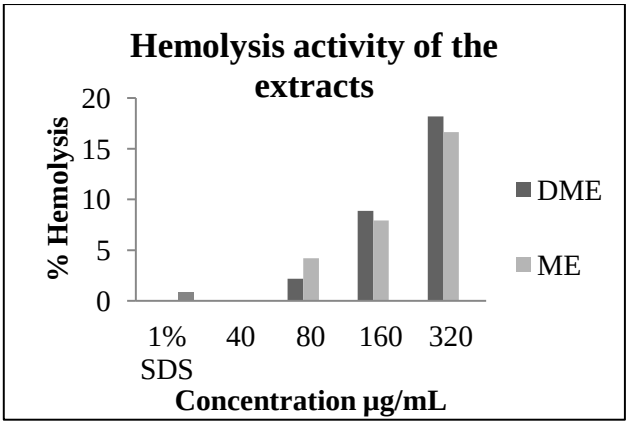

Fig 4. Determination of Percent hemolysis of DME and ME compounds in MCF-7cell lines.

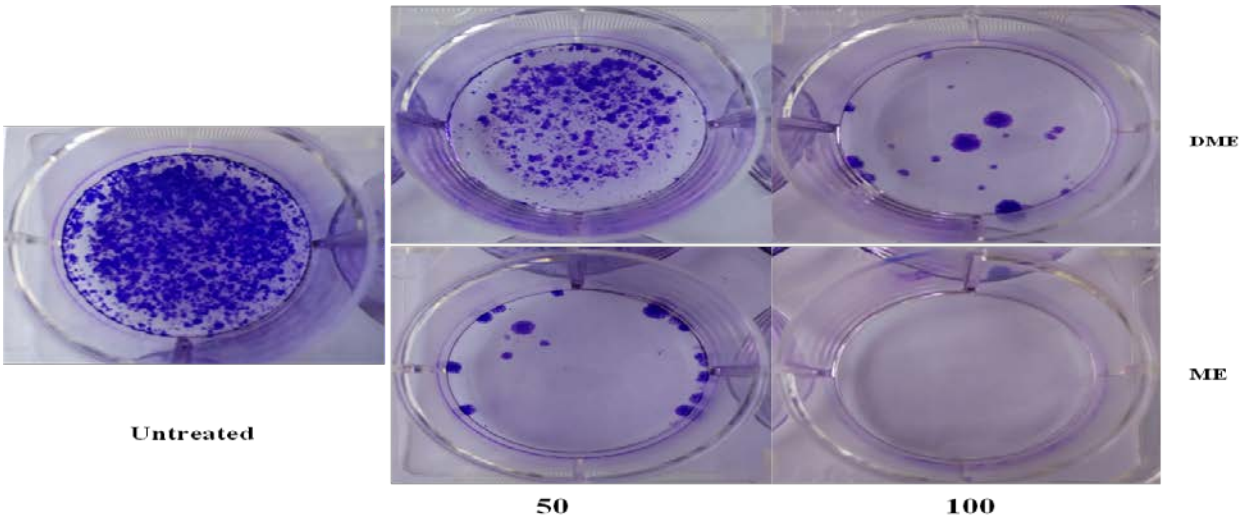

Fig. 5: Determination of Clonogenic activity of ME and DME compounds on MCF-7 culture cells

Samples		Conc.(µl)		OD 590 nm	% Inhibition	IC 50
		Control		0.6451	0.00	
DME		0.7		0.6353	1.52	35.7
		1.5		0.6145	4.74	
		3.1		0.5921	8.22	
		6.2		0.5529	14.29	
		12.5		0.5005	22.42	
		25		0.2697	58.19	
		50		0.1062	83.54	
		100		0.0551	91.46	
ME		0.7		0.6186	4.11	11.44
		1.5		0.5762	10.68	
		3.1		0.4974	22.90	
		6.2		0.4227	34.48	
		12.5		0.2877	55.40	
		25		0.1883	70.81	
		50		0.0926	85.65	
		100		0.0583	90.96	

Table 4: Formula & Summary Of Percentage Inhibition Of Dme & Me Compounds On Mcf-7 Cell Lines

Sample	Conc. µg/ml	Absorbance	% Inhibition
PBS		0.8964	0.00
1% SDS		0.1987	78.65
DME	40.00	0.89	0.00
	80.00	0.8706	2.18
	160.00	0.8111	8.87
	320.00	0.7283	18.17
ME	40.00	0.89	0.00
	80.00	0.8526	4.20
	160.00	0.8196	7.91
	320.00	0.7421	16.62

Table 5: Summary Of Hemolytic Activity Absorbance Of 1% Sds (+Ve Control) And Pbs (-Ve Control), Dme And Me Compound On Mcf-7 Cell Lines

Name	Concentration (µg/ml)	Absorbance 590nm	% Inhibition	IC_{50}
Control (1% SDS)	0.0	0.585	0.00	8.130 µg/ml
+Standard (Catechin)	0.3	0.562	3.93	
	0.6	0.548	6.32	
	1.2	0.506	13.50	
	2.5	0.429	26.67	
	5	0.254	56.58	
	10	0.151	74.19	
DME	0.0	0.585	0.00	
	3.1	0.575	1.63	86.21 µg/ml
	6.3	0.566	3.18	
	12.5	0.543	7.26	
	25.0	0.486	16.98	
	50.0	0.354	39.53	
	100.0	0.282	51.71	
ME	0.0	0.585	0.00	
	3.1	0.576	1.60	Not active
	6.3	0.572	2.27	
	12.5	0.555	5.13	
	25.0	0.509	12.92	
	50.0	0.470	19.67	
	100.0	0.393	32.82	

Table 6: Summary Of Absorbance And Percent Inhibition Of Hydroxyl Radical Scavenging Activity In Dme And Me Compounds On Mcf-7 Cell Line.

stress, depression and anxious. Majority (43%) of the elderly persons were had at fair level of coping strategies by interacting with comates, reading books, participating in social gatherings, ventilating the feelings with someone were they get disturbed. These findings were supported with the similar study has been conducted by Maddepalli Usha Rani et al., (2016) results shows that 3(3%) have mild stress, 86(86%) have moderate stress and 11(11%) have severe stress. They concluded that Elderly people residing at old age home experience moderate to severe level of stress[5]

Further study conducted by Naveen Kumar Sharma (2014) results showed that level of physiological problems among the 50 old age people are, 78% have mild physiological problems, 20% have moderate physiological problems and 2% have severe physiological problems; Level of psychological problems are, 22% have mild psychological problems, 54% have moderate psychological problems and 24% have severe psychological problems; Level of psycho-social problems are, 26% have mild psycho-social problems, 66% have moderate psycho-social problems and 08% have severe psycho-social problems and Level of overall geriatric problems are, 68% have mild geriatric

problems and 32% have moderate geriatric problems and no severe cases has been noticed. Their study results revealed that the old age people are having mild physiological, moderate psychological, moderate psycho-social problems and over all mild geriatric problems[6].

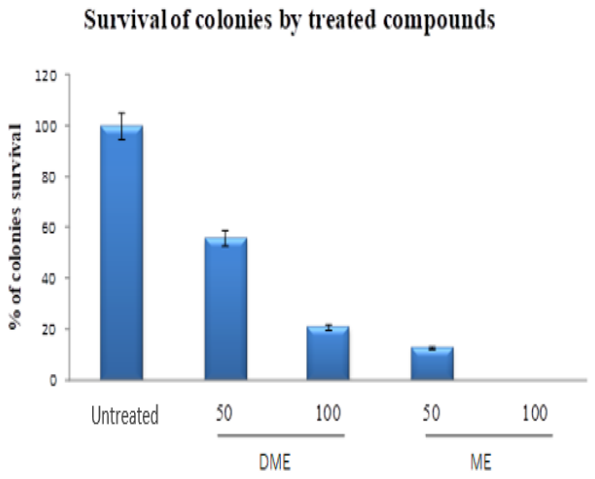

Fig. 6: Graphical representation of Clonogenic activity of ME and DME compounds on MCF-7 culture cells

Another study conducted by Geetha Mani et al., (2014) revealed that nearly 18% of the participants had high stress scores and 60% had moderate stress scores. Gender, co-living status with spouse was found to be significantly associated with stress scores. The perceived stress was high among inmates of old age homes. There is a need for organized family and social support to improve the physical and psychological health of elderly. Exploratory research studies are necessary to identify the problems among elderly, especially those in old age homes[7].
Sasmita Panigrahi, Bijayalaskhmi Dash (2015) showed that the overall stress mean score (60.6±7.28) which is 60.6% of total score, shows the moderate stress of senior citizen and the overall coping strategy mean score (68.93±5.91) which is 68.93% of total score shows that there is moderate level of coping among senior citizen[8].

CONCLUSION

Alternative medicine is becoming popular in recent times. In this brief report, we described that Cinnamaldehyde obtained from methanolic extracts of the resins of *Boswellia serrata* has shown the anti-proliferation activity on MCF-7 breast cancer cell lines, effectively. Based on preliminary observations with various assays, it is evident that Cinnamaldehyde may represent an alternative medicine which can be supplemented with chemotherapy in breast cancer patients although more breast cancer cell lines and animal models need to be used to confirm current observations.

CONFLICT OF ITEREST

The authors declare that they have no conflict of interest.

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