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In Vitro antioxidant and anti-proliferative activity of *Plectranthus amboinicus* leaves extract on MCF-7 cell line

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ABSTRACT

To investigate the in vitro Antioxidant and Anti-Proliferative Activity of Plectranthus amboinicus leaves extract on MCF-7 cell lines. Qualitative and quantitative analysis of Plectranthus amboinicus using standard methods. DPPH Radical scavenging and cytotoxicity assay carried out by standard methods. Preliminary phytochemical analysis of the methanol, ethanol, chloroform and ethyl acetate extract of Plectranthus amboinicus showed the presence of alkaloids, flavanoids, terpenoids, phenols, saponins, carbohydrates and protein. Quantitative estimation of phenol, steroid and alkaloid was carried out and the phenol was present in higher concentration than other compounds. The antioxidant activity of ethanolic extracts of Plectranthus amboinicus was investigated by in vitro study such as DPPH scavenging assay. This reveals that the ethanol extract posses a good antioxidant activity. Inhibitory concentration (IC50) for the ethanolic extract of Plectranthus amboinicus on MCF-7 cell was found to be at 32.97 determined by MTT assay. The crude ethanolic extracts of Plectranthus amboinicus has antioxidant and antiproliferative activity against MCF-7 cell lines could be due to the presence of n-Hexadecanoic acid and phytol.

Keywords: *Plectranthus amboinicus*, Cytotoxicity, Antioxidant, Qualitative and Quantitative analysis,

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INTRODUCTION

Cancer is a group of diseases caused by an uncontrolled division of cells. Its main characteristics are uncontrolled growth of cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death. Cancer harms the body when altered cells divide uncontrollably to form lumps or masses of tissue called tumors. Cancer is one of the major killing diseases, worldwide and more than 6 million people die of the disease and over 22 million people in the world are cancer patients. It is predicted that cancer incidences increasing every year in both developed and developing countries. The disease affects men and women. Thus, public and private sector institutions are focusing their research towards the development of anticancer agents. Cancer chemotherapy now plays a significant role in treating malignancies, it acts as either curative palliative care, depending upon the specific tumor situation [1].

India has a rich culture of medicinal herbs and species which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, siddha, traditional medicines but only very few have been studied chemical value. The use of medicinal plants in traditional is well known in rural areas of many developing countries. According to World Health Organization (WHO) more than 80% of the world population rely on traditional medicine for their primary health care needs and found a place in day-to-day life [2].

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecules in the protoplasm of the plant cell [3, 4]. Even with the advent of modern or allopathic medicine Velavan [2] have noted that a number of important modern drugs have been derived from plants used by indigenous people. The aim of the present work was to investigate the phytochemical constituents, antiproliferative and antioxidant activity of *Plectranthus amboinicus* extract on MCF-7 cell lines.

MATERIALS AND METHODS

Collection of plant

The fresh plant materials belong to the *Plectranthus amboinicus* were collected from Thanjavur district. The plant was authenticated by the Department of Botany, Government Arts College for Men, Kumbakonam. The plant leaves were shade dried for seven days and powered with electrical blender.

Preparation of plant extracts

Aqueous and Methanolic extract

50g of dry leaf powder of *Plectranthus amboinicus* was taken for cold maceration with aqueous (150ml) and methanolic (150ml) extract preparation. They were kept in sterile condition for 72 hours with occasional shaking. The content is filtered and evaporated using water bath for 30 minutes. The obtained plant extract was used to determine antioxidant, anticancer activity and the presence of phytochemicals.

Chloroform extract

50g of dry leaf powder of *Plectranthus amboinicus* was taken for cold maceration with chloroform (150 ml). the content is filtered and evaporated using water bath for 3o minutes. The obtained plant extract was used for screening phytochemical.

Qualitative and Quantitative analysis of phytochemical constituents

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara [8], Trease and Evans [9] and Harborne [10]. Phenols and alkaloid estimated by Malick and Singh [11] and Ferguson [12] respectively. Tannin, Saponins and Flavonoids estimated by Anonymous [13], Obadoni and Ochuko [14] and Kadifkova-panovska *et al*. [15] respectively.

ANTIOXIDANT ACTIVITY

DPPH Radical scavenging assay [12].

The free radical scavenging activity of the fraction was measured *in vitro* by 1,1 diphenyl- 2- picrylhydrazyl (DPPH) assay. About 0.3Mm solution of DPPH in 95% ethanol was prepared and 1 ml of this solution was added to 3ml of the fraction dissolved in ethanol at different concentration. The mixture was shaken and aloe to stand at room temperature for 30 minutes and the absorbance was measured at 517nm using a spectrophotometer. The percentage of the free radical scavenging activity at different concentration was determined. The antioxidant activity was expressed

$$\% \text{ disappearance} = \frac{(A_{control} - A_{Sample})}{A_{control}} \times 100$$

EVALUATION OF BIOACTIVE COMPONENTS BY GC-MS TECHNIQUE

GC-MS Analysis

GC-MS analysis of the extract was carried out with GC-MS Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) employing the following condition, Column Elite -1 fused silica capillary column (30mm ×0.25mm ID ×1 mdf, composed of 100% Dimethyl poly silaxane), operating in electron impact mode at 70 eV, Helium (99.999%) was used as a carrier gas at a constant flow of 1 ml/ min and an injection volume of 0.5 was employed (split ration of 10, 1), injector temperature 250ºC , ion source temperature was 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min), with an increase of 10ºC /min, to 200ºC

then 5C / min to 280ºC ending with a 9 minute, isothermal at 280ºC. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 40 to 550. Total GC-MS running time was 36 min.

Characterizations of compounds

Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST). The mass spectrum of the unknown compounds were compared with that of the known components stored in the NIST- library. The name, molecular weight and structure of the components of the test materials were ascertained.

***IN VITRO* CYTOTOXICITY ASSAY**

Methods

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37ºC, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

MTT ASSAY

3- [4,5- dimethylthiazol- 2-yl]2,5- diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate- dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell inhibition = 100 – Abs (sample) / Abs (control) × 100.

Nonlinear regression graph was plotted between % cell inhibition and Log concentration and IC_{50} Was determined using Graph Pad Prism software.

RESULTS AND DISCUSSION

Nature has been source of medicinal agents for thousands for years and an impressive number of modern drugs have been isolated from natural sources, many of this isolation are based on the use of the agents in traditional medicine. This plant based, traditional medicine system continues to play essential role in health care [17]. Traditional uses of plants for medicinal purposes provide a basis for the use of specific medicinal condition The plant continue to be a vital part of Western medicine, and are still considered as an important source of novel compounds in the field of drug discovery [18].

Table 1 represents the preliminary phytochemical analysis of *Plectranthus amboinicus* leaves extract. Preliminary phytochemical screening of methanol, ethanol, chloroform, ethyl acetate extracts of leaves revealed that the presence of alkaloid, flavanoid, tannins, glycosides, phenols, carbohydrates and protein. Carbohydrate and protein are present in all the four extracts. Alkaloids is present in ethanol, chloroform and ethyl acetate. Flavonoids and Tannins are present in methanol, ethanol, ethyl acetate. Phenol is present in ethanol, chloroform, ethyl acetate extract. Saponins and terpenoids is present only in ethyl acetate extract Phlobatannins, anthroquinone and cardiac glycosides are completely absent in all the extracts. Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity [19].Flavonoids have been reported to be involved in antioxidant, anti infiammatory and anti-tumor activites. Flavonoids are abundant in *Plectranthus amboinicus*. This may be partially responsible for its antioxidant activity. Further studies are required for complete understanding of intracellular mechanism of *Plectranthus amboinicus* in enhancing the antioxidant defence [20].

Plant based phenol compounds exhibit rich antioxidant activity by scavenging the free radicals generated during the normal metabolism process. This group encompasses a wide diversity of compounds. Which mainly includes, Flavonoids and pro-anthocyanidins [21]. Phenols are responsible for the variation in the antioxidant activity of the plant. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides in to freee radicals [22]. Natural phenolic compound have antitumor, antioxidant, anti mutagenic

and broad spectrum pharmacological activities in both *in vitro* and *in vivo* studies [23]. Tannins, phenolic phytochemicals, which are natural constituents of green tea, are consider to have cancer- prevention properties. Condensed tannins, isolated from black beans, did not affect the growth of normal cells, but induced cell death in a dose- dependent manner [24].

Quantitative analysis of the extract was performed and the data obtain results were represented in table 2 .We observed that the plant extract found to possess significant amount of phenols, flavonoids and steroid are lesser amount of alkaloids and saponins.

Table 1, Phytocompounds of *Plectranthus amboinicus* leaves extract

S.No	Components	Methanol extract	Ethanol extract	Chloroform extract	Ethyl acetate extract
1.	Alkaloids	_	+	+	+
2.	Flavonoids	+	+	_	+
3.	Tannins	+	+	_	+
4.	Glycosides	+	_	+	+
5.	Phenol	_	+	+	+
6.	Saponins	_	_	_	+
7.	Terpenoids	+	_	_	+
8.	Carbohydrates	+	+	+	+
9.	Protein	+	+	+	+
10	Phlobatannins	_	_	_	_
11	Anthroquinone	_	_	_	_
12	Cardiac glycoside	_	_	_	_

(+) Present (-) Absent

Table 2 Quantitative analysis of Phytoconstituents in *Plectranthus amboinicus* leaves

S.no	Constituents	Percentage(%)
1	Phenol	0.62%
2	Flavonoid	0.39%
3	Alkaloid	0.29%
4	Steroid	0.73%
5	Saponin	0.19%

Table 3 represents DPPH radical scavenging of *Plectranthus amboinicus* leaves extract respectively. 1,1- Diphenyl-2- picrylhydrazyl (DPPH), is a kind of stable organic radical. The capacity of biological reagents to scavenge DPPH radicals can be expressed as its magnitude of antioxidant ability. The DPPH oxidative assay [25] is used worldwide in the quantification of radical scavenging capacity. The antioxidant activities of plant extracts and the standard were assessed on the basis of the free radical scavenging effect of the stable DPPH free radical activity [26]. The results are expressed as the IC_{50} value (the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%). The results of the DPPH free radical scavenging assay suggest that leaves of all Cleome species have potent antioxidant property of scavenging free radicals. These species could be used as a potent source for the cancer chemo protective therapy. Antioxidants are chemical substance that donate an electron to the free radical and convert it to a harmless molecule. They may reduce the energy of the free radical or suppress radical formation or break again propagation or repair damage and reconstitute membranes.

Antioxidant based drugs and formulations for the prevention and treatment of complex disease like Alzheimer's disease and cancer have appeared during last three decades. Recent studies shown that a number of plant products including polyphenol, terpenes and various plant extract exerted an antioxidant action. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer. There is currently immense interest in natural antioxidants and their role in human health and nutrition. considerable amount of data have been generated on antioxidant properties of food plants around the globe. However, traditionally used medicinal plants awaits such screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. Numerous researches found a high correlation between antioxidative activities and phenolic content [27]

Table 3, DPPH radical scavenging activity of ethanolic extract of *Plectranthus amboinicus* leaves

S.No	Concentration (µg/ ml)	DPPH radical scavenging activity	
		Standard ascorbic acid (% of inhibition)	Leaves (% of inhibition)
1.	20	52	9.0
2.	40	60	30
3.	60	62	48
4.	80	66	63
5.	100	73	78

Table 4 represents the phytocomponents of plant leaves extract analysed by GC-MS. **Table 5** represents identified molecules from ethanolic extract of *Plectranthus amboinicus* leaves by GC-MS analysis. The extract was found to contain 14 components, of which n-Hexadecanoic acid, Thymol, 9 Octadecenal (z), 10-Heneicosene (c,t), phytol were present in higher concentration. **Table 6** represents the cytotoxic activity of ethanol extracts of *Plectranthus amboinicus* leaves on MCF 7 cells. The maximum cytotoxic effect was observed as cell death by apoptosis with its nuclear segmentation after incubation. The percentage viability was analyzed by MTT assay after treatment of chloroform extract of *Plectranthus amboinicus* at 12.5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml, 200µg/mlconcentration. IC50 value of chloroform extract was found to be at 32.97 µg/ml

Flavonoids have been shown to posses antimutagenic and antimalignant effect. Furthermore, flavonoids have a chemo preventive role in cancer through their effect on signal transduction in cell proliferative and angiogenesis. The high content of phenolic compounds are known to have direct antioxidant property due to presence of hydroxyl groups which can function as hydrogen donor. The oxidative stress has also been implicated in the pathogenesis of cancer. Many plants contain substantial amounts of antioxidant mainly flavonoid compound and can be utilized to scavenge the axcess free radicals from human body. It is reasonable to expect that high antioxidant potential to reduce free radicals in the body is due to the total phenol compound in the plant. The anticancer activity of the ethanol extract of *Plectranthus amboinicus* against Ehrlich's Ascites Carcinoma cells bearing mice was already proved by our research groups. The present study evaluated that the antitumor effect of the Ehrlich's Ascites Carcinoma cells in swiss albino mice [28, 29].

Fig 1 represents the GC-MS chromatogram of plant with eleven peaks. The phytoconstituents observed in the present study may be responsible for anti-oxidant and anticancer properties. **Fig 2 & 3** represented the MTT assay on MCF-7 cells. The graph was plotted between percentage of cell inhibition and log concentration of plant extract. A dose dependent cell inhibition was observed from the graph

Table 4, List of compounds of ethanolic extract of *Plectrathus amboinicus* leaves

S.No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: Thymol Formula: $C_{10}H_{14}O$ MW: 150	14.07	37021436	16.5849
2.	Name: 3-Octadecene, (E)- Formula: $C_{18}H_{36}$ MW: 252	20.53	785738	0.3520
3.	Name: trans-Z-à-Bisabolene epoxide Formula: $C_{15}H_{24}O$ MW: 220	20.82	2792999	1.2512
4.	Name: Dodecanoic acid Formula: $C_{12}H_{24}O_2$ MW: 200	21.56	1177527	0.5275
5.	Name: E-14-Hexadecenal Formula: $C_{16}H_{30}O$ MW: 238	26.10	5747193	2.5746
6.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	27.34	8641016	3.8710
7.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	31.44	51847784	23.2268
8.	Name: 4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate Formula: $C_{21}H_{32}O_3$ MW: 332	31.78	8097709	3.6276
9.	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	34.35	21409094	9.5908
10.	Name: 9-Octadecenal, (Z)- Formula: $C_{18}H_{34}O$ MW: 266	35.67	29054768	13.0160
11.	Name: 10-Heneicosene (c,t) Formula: $C_{21}H_{42}$ MW: 294	35.94	21928308	9.8234
12.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	36.57	14220820	6.3706
13.	Name: 1,6-Nonadien-3-ol, 3,7-dimethyl- Formula: $C_{11}H_{20}O$ MW: 168	45.34	7755375	3.4743
14.	Name: 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- Formula: $C_{30}H_{50}$	49.07	12744466	5.7093

Chromatogram

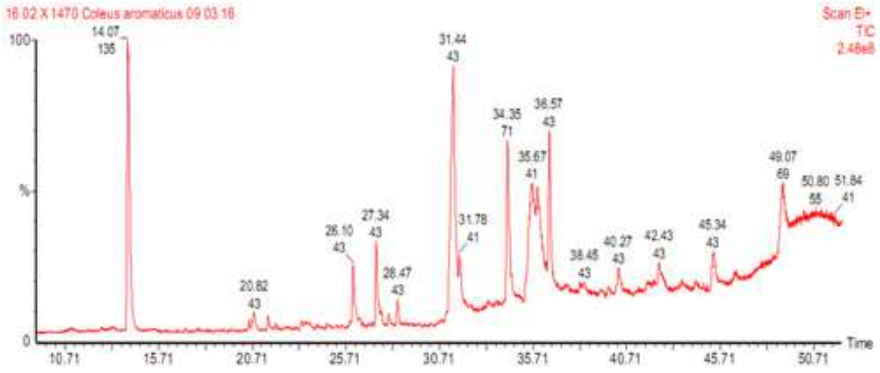


Fig 1 GC-MS report analysis of phytocompounds present in *Plectranthus amboinicus*

Table 5, Identified molecules from ethanol extract of *Plectranthus amboinicus*

Peak Name	Molecular Formula	Molecular Weight	Retention Time	Peak area	Peak area (%)
Thymol	$C_{10}H_{14}O$	150	14.07	37021436	16.5849
3,7,11,15-Tetramethyl-2-hexadecan-1-ol	$C_{20}H_{40}O$	296	27.34	8641016	3.8710
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	31.44	51847784	23.2268
4- (3,5- Di-tert- butyl-4-hydroxyphenyl) butyl acrylate	$C_{21}H_{32}O_3$	332	31.78	8097709	3.6276
Phytol	$C_{20}H_{40}O$	296	34.35	21409094	9.5908
9-Octadecenal,(z)-	$C_{18}H_{34}O$	266	35.67	29054768	13.0160
10-Heneicosene (c,t)	$C_{21}H_{42}$	296	35.94	21928308	9.8234
1,6-Nonadien-3-ol,3,7-dimethyl-	$C_{11}H_{20}O$	168	45.34	7755375	3.4743
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)-	$C_{30}H_{50}$	410	49.07	12744466	5.7093

Table 6, Anticancer activities of ethanolic extract of *Plectranthus amboinicus* against MCF-7 cell lines by MTT assay

S.No	Concentration of test (µg/ml)	% of cell inhibition	Concentration of strandard (µg/ml)	% of cell inhibition	control
1.	12.5	0.291333	0.001	14.0255	0.36
2.	25	0.291333	0.01	35.2459	0.379
3.	50	5.025492	0.1	51.27505	0.359
4.	100	11.14348	1	75.59799	0.36
5.	200	34.37728	10	92.53188	0.362

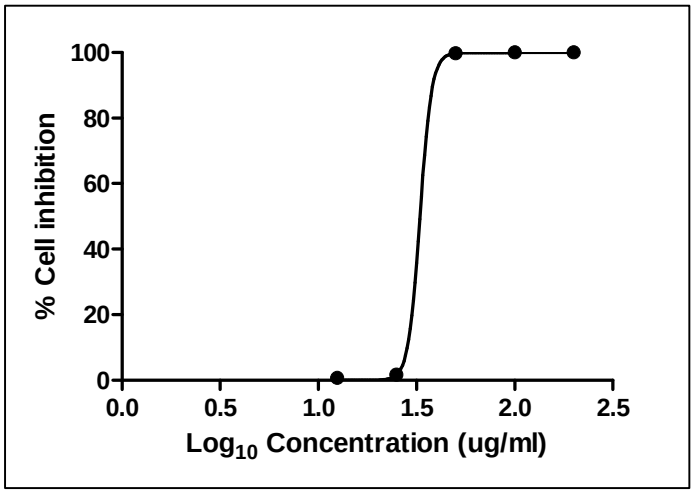
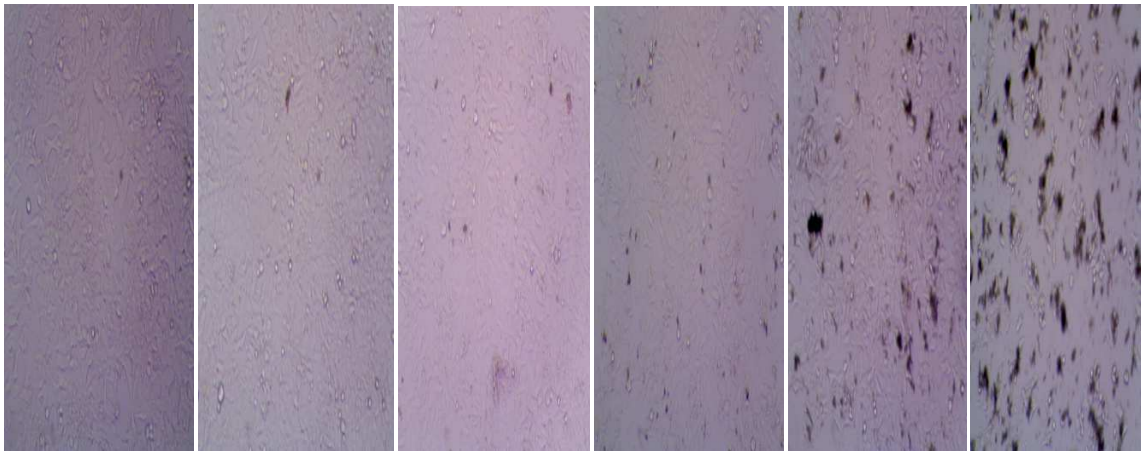


Fig 2, Effect of Anticancer activity of *Plectranthus amboinicus* on MCF-7



(a) **(b)** **(c)** **(d)** **(e)** **(f)**

Fig , 3 Cytotoxic efficacy of *Plectranthus amboinicus* leaf extract on MCF-7 cell lines

Fig 3(a) Normal MCF-7 cell line 4, (b) Toxicity at 12.5µg/ml, 5(c) Toxicity at 25µg/ml, 6(d) Toxicityat 50 µg/ml , 7(e) Toxicity at 100µg/ml , 8(f) Toxicity at 200µg/ml.

CONCLUSION

Preliminary phytochemical analysis of the methanol, ethanol, chloroform and ethyl acetate extract of *Plectranthus amboinicus* showed the presence of alkaloids, flavanoids, terpenoids, phenols, saponins, carbohydrates and protein.

Quantitative estimation of phenol, steroid and alkaloid was carried out and the phenol was present in higher concentration than other compounds. The antioxidant activity of ethanolic extracts of *Plectranthus amboinicus* was investigated by *in vitro* study such as DPPH scavenging assay. This reveals that the ethanol extract posses a good antioxidant activity. Inhibitory concentration (IC50) for the ethanolic extract of *Plectranthus amboinicus* on MCF-7 cell was found to be at 32.97 determined by MTT assay. It revealed that the crude ethanolic extracts of *Plectranthus amboinicus* has antiproliferative activity against MCF-7 cell lines. From our study it is concluded that, the phytochemicals present in the *Plectranthus amboinicus* posses significant antioxidant and anticancer activity could be due to the presence of n-Hexadecanoic acid and phytol.

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