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ON

ROLE OF PHYTOCHEMICALS AND ADVANCED MATERIALS IN CANCER PREVENTION AND RESEARCH

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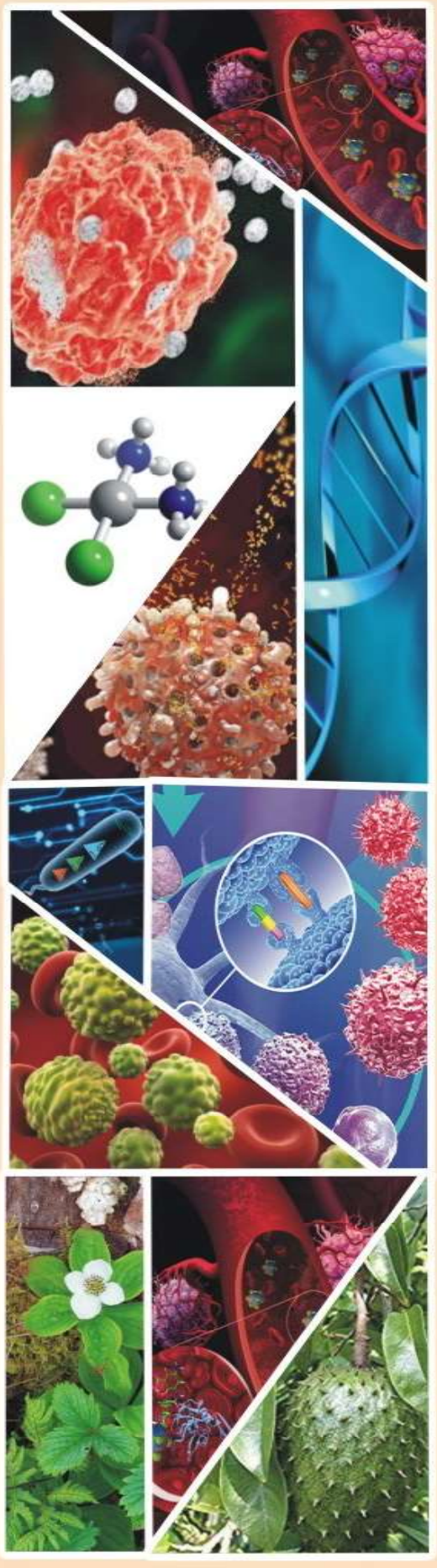

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INVITRO ANTICANCER ACTIVITY OF *Plectranthus amboinicus* LEAVES ESSENTIAL OIL AGAINST CHANG LIVER CELL LINE

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ABSTRACT

Man has always utilized natural product for different purposes, of which medicinal application is the most common.In modern days, huge attention has been directed towards recognition of plants with anticancer ability that may use effectively for human consumption. There has been rapid development of different classes of anticancer drugs with distinctive pharmacological mechanism action. There are numerous anticancer drugs derived from natural products are now commercially available and increase of modern synthetic drugs with more side effect and high cost are induced to search every possible natural products for anticancer activity. It may useful for developing countries. Therefore, based on the exhaustive literature survey, anticancer activity of essential oil of *P.amboinicus* has not been experimentally much studied. So, the present work aimed that the study in *vitro* anticancer activity using chang liver cell line.

Key words:*Plectranthusamboinicus*, Chang liver cell line.

INTRODUCTION

Plectranthusamboinicus is a tender fleshy perennial plant in the family Lamiaceae with oregano like flavor and odor, reported for many traditional uses, especially for the treatment of cough, sore throat and other nasal congestion. It is also used for a range of other problems such as infection, rheumatism and flatulence.[(1)]*Plectranthusamboinicus* or locally known as bangun-bangun, bebangun, sedingin or hati-hatihijau, is an indigenous vegetable which can be freshly eaten.[(2)] *Plectranthusamboinicus* is used to treat digestive [(3)], skin [(4)], and urinary problems, as well as respiratory illnesses such as asthma and bronchitis [(5,6)].Pharmacological activities of *Plectranthusamboinicus* have been investigated by different groups of researchers which include ethno botanical use of the plant.[(7)]*Plectranthusamboinicus* also has antitumor and cytotoxic activities.[(8,9 and 10)] In eastern Cuba it is used as herbal mixture as a traditional medicine for treating catarrhal infections.[(11)]

MATERIALS AND METHODS

Plant materials

Fresh leaves of *P.amboinicus*were collected from near pollachi, Tamilnadu, South India in the period of December. The plant sample was identified and authenticated by Dr.P.Sathishkumar, Assistant Professor, Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi and The voucher specimen was preserved in the chemistry department.

Isolation of essential oil

About 500g of fresh leaves of *P.amboinicus*was subjected to hydro distillation using Clevenger type apparatus for 3h. The leaves were immersed directly in a round bottom flask filled with water. This was then brought to boil. Vapours were condensed on a cold surface using condenser attached to it. Essential oil gets separated based on difference in density and immiscibility, is then collected and dried over anhydrous sodium sulphate and stored in vial at low temperature until analysis.[(12)]

GC-MS analysis

GC-MS analysis of the phytoconstituents of *P.amboinicus* was carried out using thermo GC –trace ultra version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non-polar column (30×0.25 mm), film thickness 0.25 µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70^0C and held for 2minutes and the temperature of the oven was raised to 260^0 C for 10minutesand raised 6^0C per minute and final temperature was 300^0C for 10minutes. The sample of 100ml was dissolved in 1ml of acetone injected with split less mode. Mass spectra were recovered over 50-500 amu range with electron impact ionization energy70eV, while injector and MS transfer line temperature were set at 230^0C and 280^0C respectively.

Identification of essential constituents

The components were identified by comparison of their mass spectra with those of NIST mass spectral library ver.2.0d, as well as on comparison of their retention time either with those of authentic compounds or with literature values.

IN VITRO ANTICANCER SCREENING

Methodology

An in vitro cytotoxicity test method was performed for the given test sample as per ISO 10993:5. The culture medium from the Chang liver monolayer was replaced with fresh medium. Test sample in duplicates were added on the cells. After incubation at 37±1°C for 18 hrs, MTT were added in all the wells and incubated for 4 hrs. After incubation, DMSO were added in the wells and read at 570 nm using photometer.

Cell treatment procedure

The monolayer cells were detached with trypin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and dilute to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100µl of these different sample dilutions were added to the appropriate wells already containing 100µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37°C, 5%CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations. (Monks et al.,1991) and (Mosmann 1983).

MTT assay

3-[4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the terazolium 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 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C 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_{10} concentration and IC50 was determined using GraphPad Prism software.

RESULTS AND DISCUSSION

The essential oils extracted by hydro distillation from the aerial parts of *P.amboinicus* were found to be pale yellow in color. Its volatile composition was analyzed by GC-MS. Totally 19 components were identified. The major componentsof essential oil wereTotarol-1-one acetate (9.08%), 3,3-Difluoro-1-methyl-2-phenylcyclopropene (7.67%), 1-(4'-Methylphenyl)-4-azidobut-1-ene(6.75%),4-(4-Methoxyphenoxy)phenyl azide (6.54%) etc., and minor components were Butanamide (2.35%), caryophyllene oxide (1.95%),pyrrolidine (1.24%),etc.,The chemical composition of essential oil of *P.amboinicus* was given in the figure and various compounds of *P.ambonicus*was given in the table.

Table- 1 GC-MS analysis of volatile composition of essential oil of *P.amboinicus*

S.NO	COMPOUND NAME	RETENTION TIME	AREA
1.	3,3-Difluoro-1-methyl-2-phenylcyclopropene	14.66	7.67
2.	Spiro[cyclohexane-1'-2H-benzopyran]	15.15	2.17
3.	Caryophyllene oxide	16.23	1.95
4.	1,2-Epoxy-1-phenyloct-7-en-3-ol	16.70	3.47
5.	1-(4'-Methylphenyl)-4-azidobut-1-ene	18.6	6.75
6.	4-Amino-2,6-dimethyl-3-pyridyl 1-adamantanecarboxylate	18.60	1.68
7.	4á-Hydroxy-9á-acetoxyaromadendrene	20.08	1.21
8.	Benzenemethanol, á,4-dimethyl-(CAS)	22.75	1.96
9.	1-formyl-5,6-dihydro-8,9-dimethoxypyrrolo[2.1-a]isoquinolino-2-carboxylate	23.14	1.24
10.	Pyrrolidine	23.89	2.53
11.	3á-Acetoxycalohastin	24.38	2.34
12.	Ethyl 1-(p-methylphenyl)-7(1H)-oxo-2,3-dihydroimidazolo[1,2-a]pyrimidine-6-carboxylate	24.67	3.37
13.	9-(3,5-dimethyl-4-methoxyphenyl)fluorene	24.97	3.96
14.	(cis)-Isokalafungin	25.38	3.40
15.	(1S)-1-methyl-1,3-dihydroisobenzothiophene	25.95	1.71
16.	Butanamide	27.38	2.35
17.	Totarol-1-one acetate	28.15	9.08
18.	4-(4-Methoxyphenoxy)phenyl azide	31.25	6.54
19.	1-chloro-4-(phenylmethyl)benzene	33.31	3.90

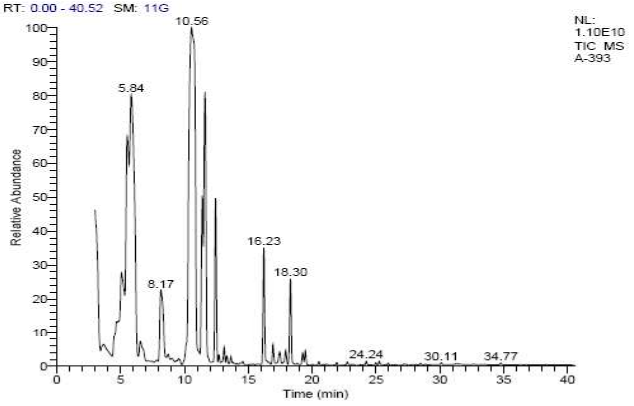

Figure-1 GC-MS chromatogram of essential oil of*P.amboinicus*

IN VITRO ANTICANCER ACTIVITY

The present study examined invitro anticancer activity of essential oil of *P.amboinicus* essential oil and the result was shown in table and figure. The essential oil was diluted 25ml, 50ml, 75ml, 100ml. The viability of cancer cells after incubation was given in the figure. The essential oil of *P.amboinicus* showed significant cytotoxicity on chang liver cell line in dose dependent pattern.

Table-2 % CELL INHIBITION OF ESSENTIAL OIL OF *P.AMBOINICUS*

S.NO	CONCENTRATION (µl)	CYTOTOXICITY (%)
1	25	7
2	50	10
3	75	44
4	100	61

Name	IC_{50} µg/ml	Name of the cell line
Essential oil	85	Chang liver

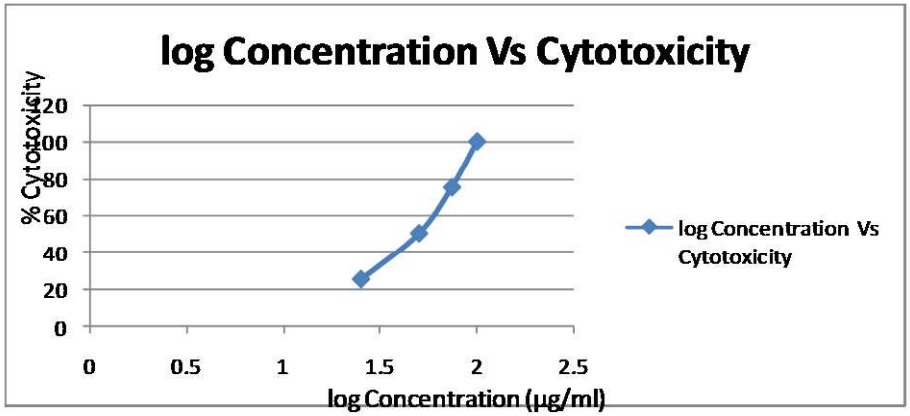

Figure -2 Invitro anticancer of essential oil of *P.amboinicus*

INVITRO ANTICANCER OF ESSENTIAL OIL OF *P. AMBOINICUS* ON CHANG LIVER CELL LINE BY MTT ASSAY

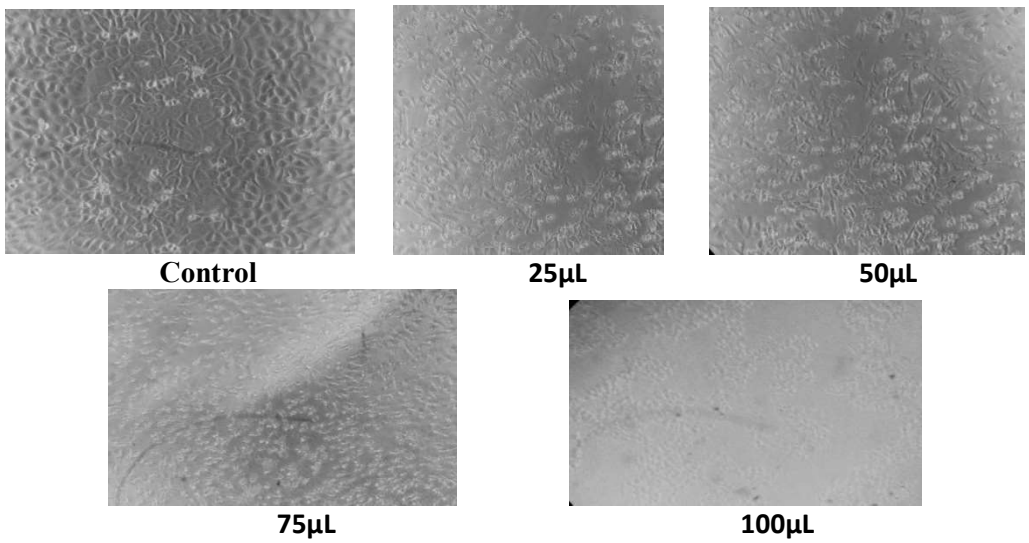

Figure-3: Invitro anticancer of essential oil of *P.amboinicus* on chang liver cell line by MTT assay

CONCLUSION

The chemical composition of essential oil of *P..amboinicus* grown in westernghats regionwas analyzed by GC-MS method.The essential oil of P.amboinicus was yellow in color, Total of 19 components were identified.Theinvitro anticancer activity of essential oil was evaluated against chang liver cell line by MTT assay.The IC_{50} value was 85µg/ml. In this present study,the essential oil of *P..amboinicus*showed a significant anticancer activity.

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