INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

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Review Article

IN VITRO CYTOTOXICITY OF ESSENTIAL OILS: A REVIEW

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ABSTRACT

Essential oils are gift by nature, which have potential bioactivity as antibacterial, antifungal, insecticidal, antioxidant, anti-inflammatory and analgesic. These are terpenes by nature and extracted by hydro-distillation method. Essential oils are found to have multiple active components which can show in-vitro cytotoxic action against various cancerous cell lines. The cytotoxicity of essential oils is believed to be due to its action upon cellular integrity leading to necrosis and apoptosis. An attempt has been made to review medicinal plants and their essential oil components which have been proven for their in vitro cytotoxic nature.

Keywords: Terpenes, Hydro-distillation, Cytotoxic.

INTRODUCTION

Nature has given indigenous gifts to the earth, one of which is plants. Plants provide us with biologically active compounds which are routinely used as remedies for various diseases. Essential oils are among those bioactive compounds which bear properties of phytomedicines. Essential oils have been reported to possess activities like antibacterial[1], antifungal[2] insecticidal[3], antioxidant[4], anti-inflammatory and analgesic[5]. Researchers all over the world are still exploring and investigating various plants to reveal and identify bioactive essential oils.

Essential oils are odorous and volatile products of various plants with a tendency to undergo evaporation when exposed to the air. Essential oils are also invariably termed as, volatile oils or ethereal oils. Essential oil is usually isolated from crude extracts using hydro-distillation in a Clevenger-type apparatus[6]. The extracted essential oil can vary both qualitatively and quantitatively with the change in climate, soil composition, plant organ, age and vegetative cycle stage[7,8]. So, in large scale essential oils of constant composition is obtained when extraction is performed under the same identical conditions. More than 500 different chemical compounds that have been isolated, purified and identified as constituents of volatile oils over the years. Analytical methods like UV-visible spectroscopy, GC-analysis, HPLC-analysis, Mass spectrometry and HPTLC are common contributors' in the phytoanalysis of volatile constituents[9]. The chemical constituents of essential oil are recognized as terpenes made up of either an isoprene unit (C_5H_8) or poly isoprene units $(C_5H_8)_n$.

Since 1990 there has been a 22% increase in cancer incidence and mortality with the four most frequent cancers being lung, breast, colorectal, and stomach and the four most deadly cancers being lung, stomach, liver, and colorectal[10].Cancer remains one of the major causes of human death worldwide[11]. In India, the statistics of the International Agency for Research on Cancer reported 635000 deaths from cancer in 2008, i.e. about 8% of all estimated global cancer deaths and about 6% of all deaths in India[12]. Between 1940 and 2002, 40% available drugs had origin from natural products and another 8% were natural product mimics[13]. Hence, exploring therapeutic agents from medicinal plants or other natural resources has become a major topic in anti-cancer drug discovery.

CYTOTOXIC ACTION OF ESSENTIAL OIL

In *in vitro* cell culture systems, cytotoxic compound interferes with cellular attachment, resulting in significant alteration in morphology, and adversely affecting cell growth rate, or causing cell death[14]. Different types of assays have been developed and are concurrently used for the measurement of viability or cytotoxicity *in vitro*[15,16,17]. Parameter measured by the cytotoxicity assays represents a proportionality with the degree of cell death in an assay well. MTT assay is mostly used colorimetric analysis and has replaced traditional radio-isotopic assays (like (^{3}H)-hypoxanthine incorporation) in evaluating cytotoxicity. Table 1 enlists different types of assay which are available for *in vitro* cell culture systems.

Presence of numerous constituents in essential oil makes it a non specific cell targeting cytotoxin. Essential oil behaves as lipophilic entity, and passes through the cell wall and cytoplasmic membrane, causing a disruption of the cellular layer comprising of polysaccharides, fatty acids and phospholipids. In eukaryotic cells, essential oils are responsible for depolarization of the mitochondrial membranes by decreasing the membrane potential, affect ionic Ca^{++} cycling[18,19,20] and other ionic channels. These along with reduction in the pH gradient, also affects the proton pump and the ATP pool. Essential oil have also been reported to increase the membrane fluidity, this results in leakage of ions, (calcium ions) proteins, thereby leading to cell death by apoptosis and necrosis.[21,22] Scanning and transmission electron microscopy observations reveal cell ultra structural alterations like swelling, shrivelling, vacuolations in several compartments such as plasma membrane, cytoplasm and nucleus. Essential oils can also function as prooxidants by affecting the cellular redox status, leading to late apoptosis and/or necrosis. The prooxidant effect functions in proteins and DNA damage thereby showing the cytotoxic effects[23].

In this article, we present the cytotoxic and cytoprotective activities of some essential oils which are used or are under investigation for their cytotoxic action over various cancer cell lines. The table 2 below depicts the plant source as well as the essential oil components which were obtained in major fraction for cytotoxic evaluation in various cancerous cell lines. During the literature research it was observed that MTT assay was mostly used for cytotoxic assay, although in one of the study of *Helichrysum gymnocephalum*, radioactive assay was applied[41].

CONCLUSION

Drug development based on potent bioactive lead compound isolation from medical plants has been a major strategy for developing new anticancer drugs from herbal source. The traditional strengths of essential oil in cytotoxicity can be assumed by the results obtained by the in vitro studies. Numerous constituents are isolated and are proved for their potential as cytotoxic agent. There is a long way to go before these studied compounds becomes a new drug, even though it is just used as botanical drug products. However, these bioactive compounds from essential oil should not remain unused and more studies should be conducted to validate the anticancer application in clinical settings. As for now the greatest challenge continues to be in translating these eminent scientific discoveries into the preclinical (quality, safety and efficacy) and clinical studies. We believe that in the future years to come, these natural products can potentially be of extreme importance in devising new drugs and providing unique ideas for the war against cancer.

Table 1: Main classes of viability or cytotoxicity assays

Main classes of viability or cytotoxicity assays		
Metabolism reductase activities • MTT assay • XTT assay • MTS assay • WST-1 assay • Resazurin based assay	**Bioluminescent ATP assays** • ATP assay	**Enzyme release-based cytotoxicity assays** • LDH activity assay • GAPDH activity assay
	Radioactive assay • (3H) Hypoxanthine incorporation	**Fluorogenic peptide based Assay**

MTT- (3-(4, 5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide)
XTT- (2, 3-bis (2-methoxy-4-nitro-5-sulphophenyl)-5-carboxanilide-2H-tetrazolium)
MTS- (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoly)-3-(4-sulfophenyl) tetrazolium, inner salt)
WST- 1-((4-[3-4-iodophenyl]-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate)
ATP- Adenosine Triphosphate
LDH- Lactate dehydrogenase
GAPDH- Glyceraldehyde-3-Phosphate Dehydrogenase

Table 2: Examples of essential oils tested for their cytotoxic action on various cell lines

Plant source	Essential Oil/Composition[#]	Target cell/Cell lines	Reference
Beilschmiedia erythrophloia	β -caryophyllene (22.6%) α -humulene (21.9%) Terpinen-4-ol (5.3%), Cis- β -ocimene (5.1%), Sabinene (5.0%) and Limonene (4.5%)	Human oral, liver, lung, colon, melanoma, and leukemic.	24
Cunninghamia lanceolata var. konishii	Cedrol (58.3%), α -cedrene (11.8%), α -terpineol (4.2%) and β -cedrene (3.5%)	Human lung, liver and oral.	25
Rosmarinus officinalis L.	1,8-cineole (27.23%), α-pinene (19.43%) and β-pinene (6.71%)	Ovarian (SK-OV-3), (HO-8910) and HeLa cells (Bel-7402)	26
Myristica fragrans	Myristicin (32.8%), Sabinene (16.1%), α-pinene (9.8%), β-pinene (9.4%), β-phellandrene (4.9%), Safrole (4.1%) and Terpinen-4-ol (3.6%).	Colon	27
Foeniculum vulgare	L-limonene (11.967%)	Breast (MCF7) and liver (Hepg-2)	28
Schinus terebinthifolius raddi.	α -phellandrene (34.38%) β -phellandrene (10.61%) α -terpineol (5.60%) α -pinene(6.49%) β -pinene (3.09%) p-cymene(7.34%) Cadinene (18.04%)	Breast (MCF-7)	29
Litsea cubeba	Citral	Lung, liver and oral.	30
Melaleuca alternifolia	Terpinen-4-ol	Murine tumour, Mesothelioma (AE17) and melanoma (B16).	31
Cymbopogon flexuosus.	Neral, Geranial Geraniol	Colon, Neuroblastoma	32
Ocimum basilicum Linn.	Methyl cinnamate (70.1%), Linalool (17.5%), β-elemene (2.6%) and Camphor (1.52%).	Cervical (HeLa), laryngeal,epithelial (HEp-2)	33
Satureja sahendica	Thymol (40%), γ-terpinene (28%), and ρ-cymene (22%)	Breast (MCF7), Kidney (Vero), Colon (SW480)	34
Satureja Intermedia	Thymol (34.5%), γ-terpinene (18.2%), and ρ-cymene (10.5%) Limonene (7.3%) α-Terpinene (7.1%) Carvacrol (6.9%) Elemicine (5.3%)	Oesophagus squamous cell (KYSE30) and human bladder (5637)	35
Anemopsis californica	Cymene, Limonene, Piperitone Thymol α-pinene, 1,8-cineole Myrtenol Methyleugenol, Isoeugenol and Elemicin	Lung (A549) Cervical (HeLa)	36
Casearia sylvestris Sw	α -pinene(4%) β−caryophyllene(18.1%) α-humulene (4.7%) Germacrene D (3.9%) Bicyclogermacrene(43.6%) Germacrene B (5.25%) Calamenene (2.3%) Spathulenol (15.9%)	Alveolar basal epithelial (A549), Colon (HT-29)	37

	Globulol (3%) α-muurolol (2.7%)		
Citrus aurantium	Limonene (96-97.7%) α-pinene (0.35-1%) β-myrcene (0.9-1.4%)	Colorectal (LIM1863)	38
Croton matourensis *Croton micans*	Fenchyl acetate (19.5%), Methyleugenol(14.2%), Isoelemicine (11.3%), Elemicine (7.6%), Spathulenol (6.9%) and Valencene (5.8%) ***Flower extract:*** Fenchyl acetate (41.6%), Caryophyllene (12.6%), Cubebene (5.0%), Caryophyllene (5.5%), Cubebene (5.3%), Elemene (4.7%) and Valencene (4.6%). ***Leaf extract:*** Fenchyl acetate (25.3%) Caryophyllene (20.7%), Selinene (12.8%) and Bourbene (9.3%)	Colon (LoVo), colon (X-17), HeLa (cervical cancer) Colon (LoVo), Colon (X-17), HeLa (cervical cancer)	39
Eucalyptus benthamii	α-pinene (36.82%), Globulol (20.54%), Aromadendrene (15.94%), and γ-terpinene (5.51%).	T leukemia cells, murine macrophage tumor (J774A.1), and HeLa (cervical cancer)	40
Helichrysum gymnocephalum	1,8-Cineole (47.4%), Bicyclosesquiphellandrene (5.6%), γ-curcumene (5.6%), α-amorphene (5.1%) and Bicyclogermacrene (5%)	Breast (MCF-7)	41
Zanthoxylum zanthoxyloides *Zanthoxylum leprieurii*	Citronellol and Geraniol E- β-ocimene	Liver colon, breast , prostate Liver, colon, breast, Prostate.	42
Libanotis transcaucasica	Germacrene B (20.2%) Isospathulenol (11.0%), Germacrene D (9.2%) and Kessane (5.5%)	HeLa, Colon (LS180), Breast (MCF-7) and lymphoblasts (Raji)	43
Marrubium vulgare L.	β -citronellol (9.90%) Citronellyl formate (9.50%) Germacrene-D (9.37%) γ -Eudesmol (11.93%)	cervical (HeLa)	44
Dorema ammoniacum D.Don	(Z)-Ocimenone (22.3%) (E)-Ocimenone (18.1) β –Cyclocitral (9.9%) Curcumene (6.4%)	Human fetal skin fibroblast (HFSF), colon (SW480), Breast (MCF) and Human fetal liver fibroblast (HFLP)	45
Ricinus communis L	α-pinene (16.88%), Camphor (12.98%) and Camphene (7.48%). 1,8-cineole (30.98) α-thujone (31.71%)	Cervical	46
Thymus broussonettii	Borneol (33.92%) Thymol (37.11) Carvacrol (5.26)	Murine mastocytoma (P-815), human chronic myelogenous leukemia (K-562), acute T lymphoblastoid leukemia (CEM), human breast	47

		adenocarcinoma (MCF - 7)	
Tridax procumbens	α-pinene β-pinene Phellandrene and Sabinene	Melanoma cell line (B16F-10)	48
Melaleuca alternifolia	Terpinen-4-ol	Mesothelioma (AE17) and Melanoma (B16)	49

Components with higher % is mentioned.

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