

Research Article

Effect of Ocimum on Cervical Intraepithelial Neoplasia (CIN) Pre Cervical Cancer

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Abstract

Diethylstilbestrol is a synthetic estrogen hormone which is useful as hormone replacement therapy, but it also can trigger the abnormal cells growth that will develop into cervical cancer. Cervical cancer is associated with invasion and metastasis potential where the gold standard is a histopathological examination. [1][2] Histopathological examination is a method of seeing *Cervical Intraepithelial Neoplasia* growth. There is no therapy needed at the CIN1 medium, 80% can return to normal. CIN II & III can develop into cancer cells (true cervical cancers precursors). CIN or pre-cancer therapy is more effective and efficient, and also low cost before it develops into malignant cancer cells. Accurate diagnosis and prediction of malignancy are important issues in clinical management, including biomarker identification for CIN diagnosis. CIN screening is an effective method to identify the presence of cancer earlier to decrease the mortality. CIN that has been treated can regrow/recur by some trigger factors such as age, menopause, and surgery. Ocimum has an anti-cancer effect through the caspase-3 induction which stimulates PARF to release cytochrome-c. PARF induces apoptosis in cancer cells through ROS increase. Ocimum has antioxidant activity, protects cell damage, and acts as a *scavenger highly reactive free radicals*. The results of the study group who received 100% diethylstilbestrol had Cervical Intraepithelial Neoplasm (CIN). 40% at CIN2 and CIN3 Stadium, and 20% at CIN1. Chi-square test results showed that there was a significant effect between the administration of *diethylstilbestrol* and the occurrence of CIN in p-value <0.05. Then, there was no significant effect between the administration of Ocimum extract and CIN stadium with p-value > 0.05.

Keyword: Pre, Cervical Cancer, Diethylstilbestrol, CIN, Ocimum, Perinatal, Wistar Rat, In vivo

Introduction

Based on the WHO report in 2015, it was found more than 528,000 new cervical cancer cases. About 80-90% are in developing countries. Cervical cancer is in second-ranking in all cancer cases in the world.[1]. In 2012, almost 266,000 women in the world died of cervical cancer. The number of people with cervical cancer is predicted to increase 1-5 times in 2030. [1]–[3] Cervical cancer is 98% related to the *Human Papilloma Virus* (HPV). HPV infection does not develop into cervical cancer as a whole because endogenous and exogenous factors are indicated to affect the process of cervical cancer[1], [4]. Some risk factors for cervical cancer are (1) socio-demographic; age, socioeconomic status, (2) factors of sexual activity; the age of first sexual intercourse. There is no therapy needed at the CIN1 medium, 80% from this cases can

return to be normal. [5]. Women with CIN II & III need therapy such as a laser, *cryotherapy*, *LEEP (Loop Electrosurgical Excision Procedure)*, and laser conization. CIN II and III cases can develop into true cervical cancers precursors within 12 months.[5]

Almost 330,000 CIN cases have been found in Europe, 50% of them are CIN2 and CIN3. 1.5 per 1000 women in developing countries were found diagnosed with CIN2 and CIN3, the highest incidence was 25 to 29 years. [6]. CIN does not cause specific symptoms. Accurate diagnosis and prediction of malignancy are important issues in clinical management, including the identification of biomarkers for CIN diagnosis.[5]

CIN *screening* is an effective method to identify the presence of cancer earlier to decrease the mortality. CIN that has been treated can regrow/recur by some trigger factors such as age, menopause, and surgery.[7].

Pre-cancer therapy is more effective and efficient, and also low cost before it develops into malignant cancer cells.[8][9]. Ocimum contains a number of important compounds such as 1-8 cineol compounds, arigin, anetol compounds, flavonoids, boron, stigma sterol, eugenol, beta-carotene, magnesium, tryptophan, and volatile. Ocimum has an anti-cancer effect through the caspase-3 induction which stimulates PARF to release cytochrome-c[10]. Ocimum has antioxidant activity, protects cell damage, and acts as a *scavenger highly reactive free radicals*. Ocimum metabolites, namely; alkaloids, polyphenols, triterpene flavonoids. These metabolites cause apoptosis by modulating p53, Bcl-2, and caspase. [11].

Ocimum leaves contain a highly active substance eugenol, by giving eugenol 100 mg/kg BB with a frequency of 3 times a week in mice induced *N-methyl-N'-nitro-N-nitrosoguanidine* (MNNG) gastric cancer for 26 weeks, causing tumor cell apoptosis and it does not cause apoptosis in the control group.[12]. Rosmarinic acid in Ocimum leaves inhibits the proliferation of tumor necrosis factor- α , inhibits the G0 – G1 and G1 – S phases.[13]. The content of terpene in Ocimum (β -element) increases H1 (Histon1) which will inhibit transcription. Histone is a protective protein of DNA structure. [14]. Research question. [15]. Ocimum leaves have an anti-cancer effect through induction of apoptosis in *cell line* (HeLa) cervical cancer. The effect of its anti-cancer is by decreasing Bcl-2 expression and increasing Bax, cytochrome-c, and caspase-3 expression.[16]. Ocimum leaves have an anti-cancer effect through induction of apoptosis in cell line (HeLa) cervical cancer. The effect of its anti-cancer is by decreasing Bcl-2 expression and increasing Bax, cytochrome-c, and caspase-3 expression.

The results of various studies above showed that Ocimum exposure causes disruption in abnormal cell growth which will activate the proapoptotic signal. Based on the explanation above, an *in vivo* study was conducted on the effect of Ocimum leaves extract in CIN stadium of pre-cervical cancer mice models after induction of diethylstilbestrol (DES)[17]

Materials and Methods

Solution Preparation and Administration of DES

DES was obtained from SIGMA-ALDRICH.Co.3050 Sprunce Street, ST Louis M @, 63103, USA 314-7715763, which contains >99% synthetic estrogen content. Preparation of DES 1500 dose μ gram / kg BB to see the presence of CIN was done by dissolving DES in a solution of corn oil and it was given a single dose

subcutaneously in 3-day-old Wistar rats, 10 grams in weight, 0.01 ml.

Extract Preparation and Administration of Ocimum

The type of Ocimum was used namely Ocimum Citriodorum which is widely available in Indonesia (Java). The extract uses Ocimum leaves from BALITRO, Bogor, West Java. The procedure of making extracts consists of 2 stages. The first stage was making Ocimum Citriodorum powder and the second stage was making Ocimum Citriodorum herbal extract by maceration with variations in the composition of ethanol and water. [18]. Then the Ocimum extract was given in a sonde to Wistar rats according to the dosage. For untreated mice, the NaCMC solution was given as a placebo.

Histopathology Examination

The cervical tissue was fixed by a frozen section method by soaking the tissue with an ethanol solution to remove liquid from the tissue, followed by a solution of toluene or xylene, then with paraffin. The final result was formed paraffin block, where it was then sliced thinly with a special microtome knife. The thin slices were then placed on a glass object, fixed further with the same solution as the chemical fixation method. The next step was painting. The tissue was painted with special dyes. Finally, after the tissue was fixed and colored, tissue in the glass object can be read under a microscope and epithelial cells can be seen in the cervical tissue.

Observation Time

Rats were divided into 4 groups, each group was consisted of 5 rats. Group 1 was the negative control group, only given a placebo in the form of 1% NaCMC. Group II was given DES at a dose of 1500 μ gram / kgBB without Ocimum, group III and IV was given a dose of 1500 μ gram / kgBB with Ocimum extract, each dose of 600 mg / kgBB and 800 mg / kgBB, the four groups then euthanized at 49 days or 14 days after administration (given starting at 35 days), the cervical epithelium was examined in stadium CI observation for each group, the results were compared to controls.

Data Analysis

This research was an experimental research which was conducted *in vivo*.

Data analysis in this research used chi-square test.

Results and Discussions

The results of CIN description study on the cervical epithelium group given by DES and the group that received DES with Ocimum can be seen in the figure below.

Figure 1. CIN Description in the treatment and control groups

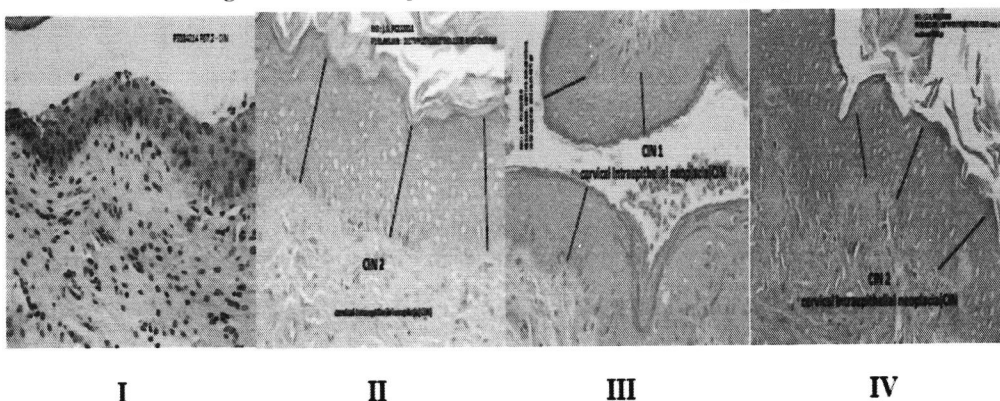
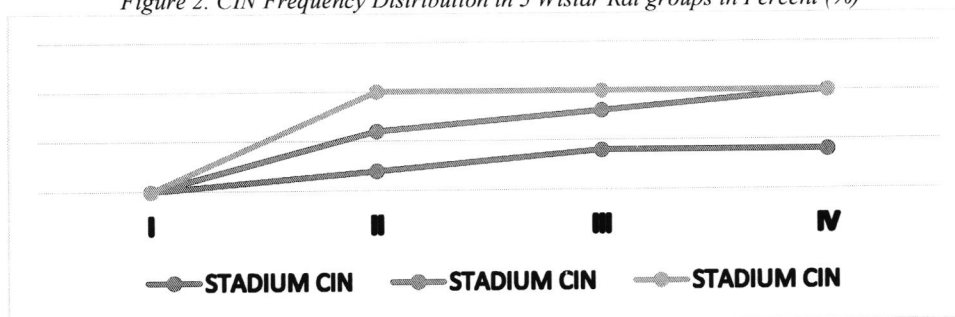


Figure 2. CIN Frequency Distribution in 5 Wistar Rat groups in Percent (%)



Group I, all epithelial cells grew normally. Meanwhile, group II, III, and IV cervical epithelial cells had abnormal growth (dysplasia). CIN was found in group I, II and III. CIN Stadium was seen in group IV, it was not found CIN3 stadium. This group IV received Ocimum extract at a dose of 800 mg/KgBB. The result of the *chi-square* test showed that there was a significant effect between the mean of the control group (only given DES) and the occurrence of CIN with $p\text{-value} = <0.01$.

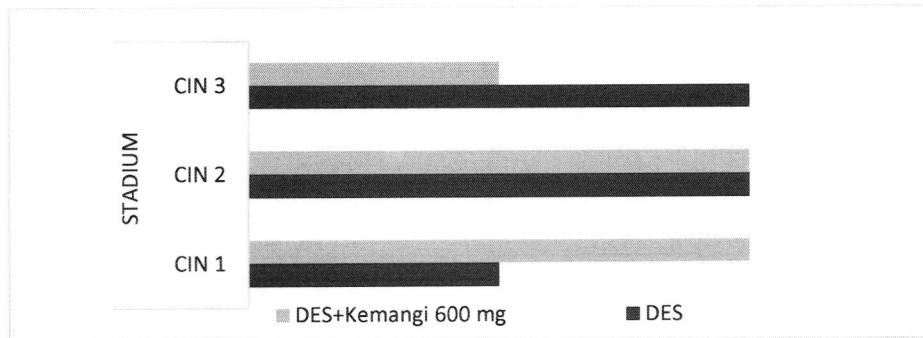
The results showed that administration of DES 100% caused cervical epithelial dysplasia (CIN). [19] and stimulates the formation of the abnormal cell. DES works to interfere neuroendocrine, inhibits the release of the hormone gonadotrophin-release hormone (GnRH). DES will reduce the level of *kiss peptin1*, mRNA will reduce GnRH stimulation in neurons stimulated by *kiss peptin*. *Kiss Peptin* is a regulator or regulates the production of GnRH. *Kiss peptin*, also regulates ovulation, estrus cycles, sex differentiation, and affects puberty. [20].

DES causes proliferation of vaginal epithelium through estrogen receptors. [21] Estrogen receptors are

in the nucleus, estrogen bonds, and estrogen receptors increase transcription in the target gene. Activation of these target genes will increase the tissue response, followed by the increasing of protooncogene including mRNA *c-fos*, *c-jun*, and *c-myc*. In the vaginal epithelium, the increasing of protooncogene *c-fos*, *c-jun* is almost 4-fold. [22]

DES or diethylstilbestrol is a synthesis of estrogen, has the chemical formula is $C_{18}H_{20}O_2$. DES is used to prevent miscarriage or premature labor. In 1946-1971, around 2 to 4 million individuals were exposed to DES during their pregnancy.[13] Women exposed to DES during pregnancy causes a disorder called *DES daughters*. *DES daughters* show reproductive tract abnormalities in the form of vaginal and cervical adenoid followed by the development of columnar epithelium in the cervix and vagina. *DES daughter* has a risk of developing *cervical clear cell-adenoma*. Adenosis cervical vaginal is a precursor of adenocarcinoma. [5]

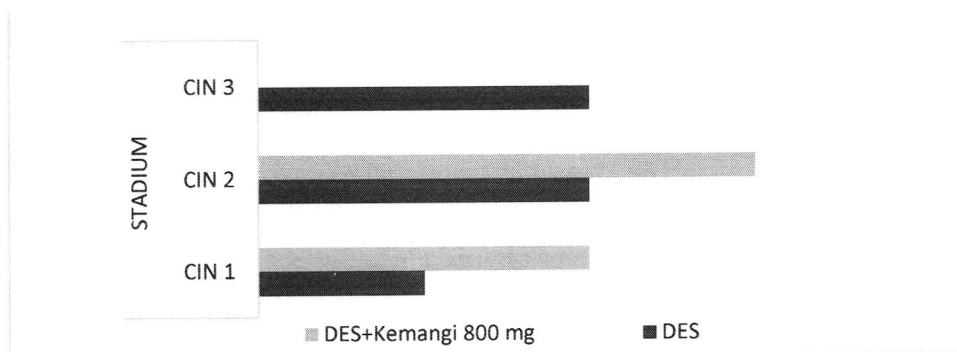
Figure 3. CIN Stadium, the Rat got DES and DES with Ocimum 600 mg/kgBB



The *Chi-square* test results showed, there was no significant difference in the mean group which was given DES and Ocimum extract, with a dose of 600mg/KgBB compared to the control group with the p-value = >0.05. There was no significant difference in the statistical test results because the number of samples was relatively small (5 rats) or possibly because of the CIN stadium was not homogeneous/different, thus it was

difficult to observe the effect of Ocimum on non-homogeneous CIN stadium. It recommends, in the future research to see the effect of Ocimum on pre-cancer, the group of rat must be at the same CIN stadium. However, the results of the study illustrate that the group was given DES and Ocimum extract doses of 600 mg/KgBB, the frequency of CIN3 stadium was lower than CIN1 and 2.

Figure 4. CIN Stadium, the Rat got DES and DES with Ocimum 800 mg/kgBB



Chi-square statistic results were obtained $p > 0.05$, which means, there was no significant difference the mean of groups were given DES and Ocimum extract before and after administration of 800mg/KgBB with CIN medium with p-value = > 0.05. (0.08). There was no significant difference in the statistical test results, it possible because of the lack of doses, thus the future research, it should increase the doses. The number of samples was relatively small (5 rats) or possibly because of the CIN stadium was not homogeneous/different, thus it was difficult to observe the effect of Ocimum on non-homogeneous CIN stadium. Giving Ocimum leaves extract doses of 800 mg/Kg BB in CIN, all rat groups

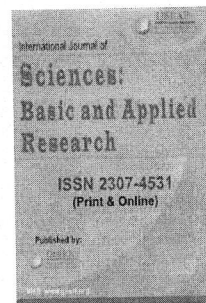
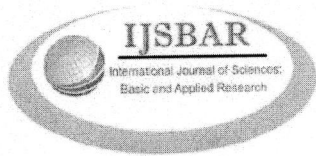
were given DES was positive CIN1 stadium, 2, and 3 but DES group with Ocimum extract, it was not found CIN3 medium.

Conclusions

The results of this study, there was a significant effect between administration of diethylstilbestrol with CIN ($p < 0.05$) and there was no significant correlation between the effects of Ocimum extract dose of 600mg/Kg BB and a dose of 800mg/KgBB on CIN with $p > 0.05$. This condition may be caused by the number of small samples or inappropriate dose given to inhibit the growth of abnormal cells in the cervix. However, there was no CIN3 found in the 800mg dose group where Ocimum had an anti-cancer effect.

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Ocimum Basilicum as Alternative Natural Cancer Care

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Abstract

Cancer is the most dreadful disease worldwide in terms of morbidity and mortality. The exact cause of cancer development and progression is not fully known. *Ocimum Basilicum* (OB) or basil contains citric acid, essential oils, 1-8 seneol, arigin, anetol, flavonoid, boron, stigmasterol, eugenol, beta-carotene, magnesium and tryptophan. Works as an anticancer through phenolic compounds (caffeine acid, p-kumarat acid) which is an antiproliferative agent, which will increase the number of non-apoptotic cells in the synthesis phase in the cell cycle and decrease the number of non-apoptotic cells in the G2/M phase. While caffeine acid may cause an increase in FasL, which is a receptor of death (Fas). The existence of a complex between FasL and Fas can induce signaling that induces cell death. Phenolics have also been shown to decrease the Bcl2 antiapoptotic protein, so apoptosis does not work. Increased Bcl2 expression will be followed by an increase in Bax, which is produced because cancer cells may mutate in the p53 protein, triggering a dimer between the two and triggering apoptosis through Fas.

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OB extract, containing flavonoids that have an effect as a chemopreventive agent by protecting lymphocytes from cancer induction. Another compound is the essential oil (terpenoid) that can inhibit tumor by improving the performance of Human Natural Killer in immune system (immunostimulan), so as to destroy tumor cells and increase macrophage cells that act as the phagocytes of damaged cells. In this review will be discussed about the role of *OB* in the natural treatment of cancer and the working mechanisms involved in it.

Keywords: *Ocimum Basilicum*; Anti-cancer; anti proliferative compound; working mechanism.

1. Introduction

Cancer is a disease characterized by out-of-control cell growth leading to spread of abnormal cells to other body parts by local invasion and/or distant metastasis. It is one of the major and growing public health problem, currently accounting for over 12% deaths globally. Cancer is a major health problem that can debilitate and destroy human lives. One out of every four deaths in the U.S. is caused by cancer. Over \$124.6 billion was spent in direct medical costs for the 13.7 million cancer survivors and 1.5 million newly diagnosed cancer patients in the U.S. in 2010 [1]. The World Cancer Report showed that cancer rates would increase at an alarming rate in the global scope. In this report, cancer rates could further increase by 50% to 15% million new cases in the year 2020 [2]. Numerous natural compounds have been extensively investigated for their potential for cancer prevention over decades. Phytochemicals are compounds found in plants i.e *curcumin*, *capsaicin*, black tea and *ocimum basilicum* in experimental systems and clinical trials, they protect people from environmental and ingested carcinogens by arming our antioxidant enzymes, enhancing DNA repair pathways and have direct effects on the fundamental hallmarks of cancer progression and metastasis. Chemoprevention is one of the cancer prevention approaches where in natural/synthetic agents are prescribed with the aim to delay or disrupt multiple pathways and processes involved at multiple steps, i.e., initiation, promotion, and progression of cancer. Majority of human cancers are caused by environmental and life-style factors. The etiology of all cancers is associated with inherited genetic aberrations (5%-10%) and acquired genetic abnormality (90%-95%) caused by exogenous and/or endogenous environmental agents [3].

Ocimum Basilicum (*OB*) or basil in Indonesia is known by the name of basil, except in Sulawesi known as amping [4], grows in the tropical region and is a herb plant erect or bush, branched a lot, with height 1.3-1.5 meters, has a distinctive fragrant sourced from citric acid, especially the flowers and leaves. Studies have shown many pharmacological effects in several diseases, with potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties [5]. *Ocimum* consists of several types: *Ocimum basilicum*, *Ocimum sanctum*, *Ocimum gratissimum* and *Ocimum americanum*. Basil includes vegetables rich in provitamin A (in 100 grams of basil leaves containing 5,000 SI of vitamin A), calcium and phosphorus, (in 100 grams of basil leaves containing 45 and 75 mg of phosphorus). Other active ingredients contain phenol, terpenoids, alkaloids, glycosides, tannins, caffeic acid, p-kumarite acid, myresin, routine, quercetin. All herbs contain volatile oils comprising: 1,8-Sineol, p-Cymene, Limonen, Linalool, Methylcaviol, Methyl cinnamate, Pinen, Safrol, alpha-Terpinent [6]. Based on the results of the research, the evaporated oil has an anti-bacterial activity that has been tested with *S. aureus*, *S. enteritidis* and *E. coli* and its effective antifungal activity against *C. albicans*, *P. notatum*, and *Microsporeum gypseum*. *Kamfor*, *d-limonen*, *myresen*, and thymol have activity as antirepellant, with ability to kill insects up to

90% at concentrations of 113-283 ppm. Basil has also been used as anti-ekspektoran. Fresh basil leaf and stem extract contain: *cirsilineol*, *circimaritin*, *isothymusin*, *apigenin*, *rosmarinic acid* and *eugenol*. Besides it also contains sesquiterpenes and monoterpenes such as *bornyl acetate*, α and β *pinen*, *kamphene*, *cholesterol*, *stigmasterol* and β -*sitosterol*. *Eugenol* contained in fresh basil leaves and extracts have antioxidant activity and work by slowly excreting the oxidation product. Its antioxidants protect cells from damage by acting as scavengers of highly reactive free radicals [7].

2. Discussion

2.1. Cancer

Cancer is the most dreadful disease worldwide in terms of morbidity and mortality. The exact cause of cancer development and progression is not fully known. There is increasingly convincing evidence to show that plant phytochemicals have significant benefits for humans. Not only do they improve our daily lives by helping our food taste, smell and look appetising, they also reduce our risk of cancer and help people living with and beyond treatments [8]. Majority of human cancers are caused by environmental and life-style factors. The etiology of all cancers is associated with inherited genetic aberrations (5%-10%) and acquired genetic abnormality (90%-95%) caused by exogenous and/or endogenous environmental agents [3].

2.2. *Ocimum basilicum*

Ocimum basilicum L. popularly known as Tulsi in Hindi and "Sweet Basil" in English is one of the sacred herbs for Hindus in the Indian subcontinent. And India has one of the oldest, richest, and most diverse cultural living traditions associated with the use of medicinal plants [9]. Several types of basil have become commercial commodities including *Ocimum basilicum*, *Ocimum sanctum*, *Ocimum gratissimum* and *Ocimum americanum*. Studies have shown many pharmacological effects in several diseases, with potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties [5]. Some types of *ocimum* can be seen in Figure 1 below.



ocimum Basilicum

Ocimum Gratissimum

Ocimum Sanctum

Ocimum citriodorum

Figure 1: Various Ocimum Type [10]

2.3. Ingredient substance *Ocimum basilicum* and its role as anti-cancer

The chemical structure of the active ingredients found in basil can be seen in Figure 2 below.

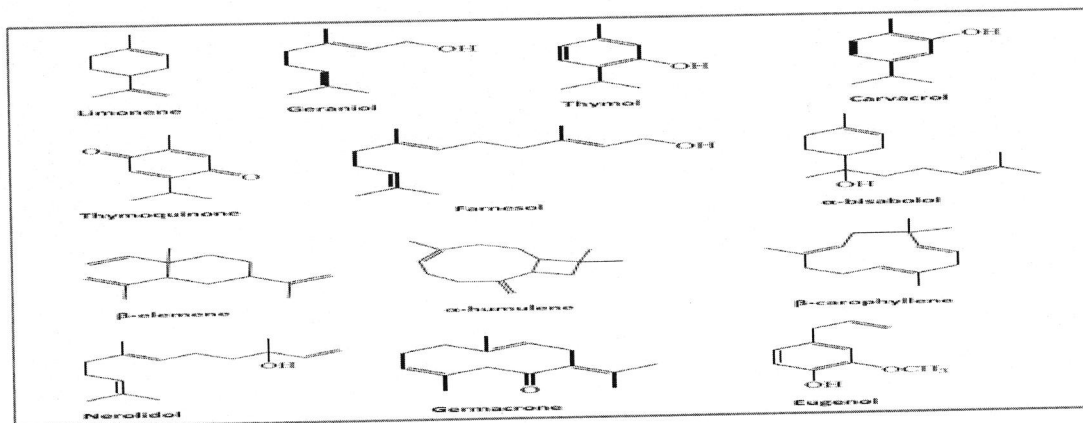


Figure 2: The chemical structure of the active ingredient found in basil [11]

Administration of basil extracts increases antioxidant enzymes such as SOD, CAT, GSH, GPx and lipid peroxide downtake [7]. *Lamiaceae* family contains *monoterpene* and *sesquiterpenes*. *Monoterpene* with its derivatives: *camphor*, *limonen*, *thymol*, *citrit*, *geraniol*, and *linalool*. *Phenols* and *flavonoids* consist of: *cinnamic acid*, *caffeic acid*, *sinapic acid*, *ferulic acid*, and *rosmarinic acid* as the main antioxidants, free radical scavengers and metal chelators. Rosmarinic acid works to inhibit the proliferation of tumor necrosis factor- α (TNF- α), and inhibit the G0-G1 and G1-S phases in cell division [12]. The role of basil as anti-cancer can be seen in the following chart below:

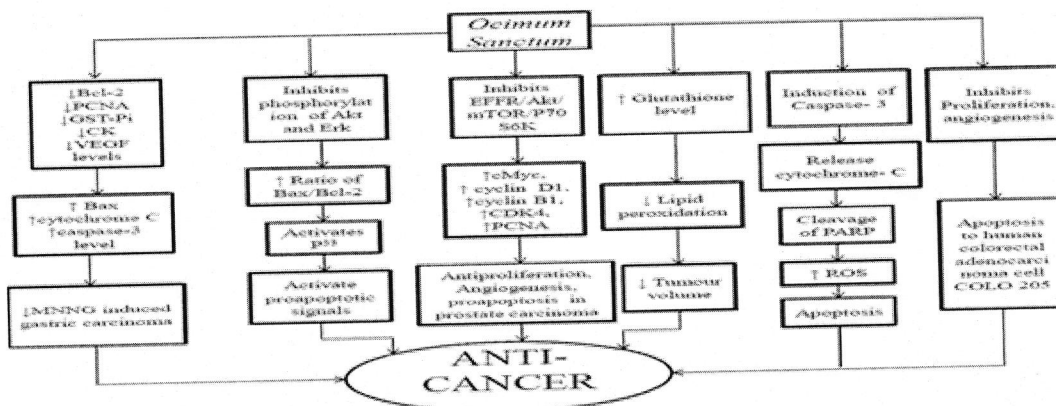


Figure 3: The basil mechanism (*Ocimum sanctum*) as anti-cancer [7]

The administration of basil leaf extract (*ocimum sanctum*) in ethanol has anti-cancer effects acting on N-methyl-N-nitroguanidine induced by gastric cancer by decreasing Bcl-2 expression regulation and increasing regulation of Bax, cytochrome C and caspase-3 expression. Basil leaves also have anti-tumor effects through the

phosphorylation of Akt and extracellular signal regulated kinase (ERK) and increased Bax protein proapoptosis / suppression antiapoptosis protein Bcl-2 ratios in p53 activation by regulating activation of proapoptosis pathway in animal models of lung cancer model And in A549 human cancer cells. Basil oil (Tulsi leaf) is reported to cause cytotoxic effects and apoptotic activity in COLO205 colorectal adenocarcinoma cells through proliferative barrier and suppress angiogenesis. Vicenin-2 in basil leaves is a flavonoid that has anti-proliferative, anti-angiogenesis and pro apoptotic effects on prostate carcinoma cells by inhibiting EFGR (epidermal growth factor). The administration of *Ocimum sanctum* (200 mg/kg, once daily) egg basil in arsenic-exposed mice for 4 months (100 ppm in drinking water) caused low glutathione (GSH) levels and increased reactive oxygen species (ROS) in the blood. The activity of superoxide dismutase and catalase in the organs: liver, kidney and brain decreased. After exposure to arsenic, then the rats were given basil genus extract *ocimum sanctum*, significantly improved levels of GSH and ROS in the blood [13]. Another basil genus *Ocimum gratissimum* has the effect of inhibiting the proliferation of cancer cells through several mechanism signals. In colon cancer cells (HT-29, P53 null) and prostate cancer cells (PC-3; P53 null) work to decrease cyclin D1 and induction of p21 expression in HT-29 and PC-3 cancer cells after 24 hours of extracts *ocimum* [14]. The administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) causes gastric cancer. Gastric cells experience proliferation and angiogenesis with evasion of apoptosis, as revealed through upregulation proliferating cell nuclear antigen (PCNA), glutathione S-transferase-pi (GST-pi), Bcl-2, cytokeratin (CK) and vascular endothelial growth factor (VEGF) And Bax downregulation, cytochrome C and caspase 3 protein expression. The administration of basil leaf extract (*ocimum sanctum*) extracted in ethanol decreases the effect of MNNG that causes cancer in the stomach. Reduces expression of PCNA, GST-pi, Bcl-2, CK, VEGF, and Bax overexpression, cytochrome C, and caspase 3 [15]. *Ocimum basilicum* leaves, used as anticancer through phenolic compounds (caffeine acid, p-acid kumarat) are in large numbers. The *ocimum* extract contains flavonoids that have an effect as a chemopreventive agent that protects lymphocytes from cancer induction, as well as terpenoid content that can enhance macrophages in their role as phagocytes of damaged cells. The administration of this extract in ethanol has an anti-cancer effect on N-methyl-N-nitrosoguanidine which in gastric cancer induces me through downregulating Bcl-2 and upregulating 3 expression [14].

Based on the above explanation of basil leaves can be used as a solution of traditional cancer drugs. At this time traditional medicine is more desirable by the community, because prefer healthy with a relatively cheap and safe cost. In addition to the side effects of traditional medicine, it is believed to be smaller and even absent when compared to the use of modern chemicals or drugs.

3. Conclusion

Phytochemicals are compounds found in plants, i.e: *Ocimum basilicum*, has the effect of inhibiting the proliferation of cancer cells through several mechanism signals. Which are responsible for the colour, taste and aroma of foods. Over and above these pleasant attributes, they protect people from environmental and ingested carcinogens by arming our antioxidant enzymes, enhancing DNA repair pathways and have direct effects on the fundamental hallmarks of cancer progression and metastasis. Chemoprevention by dietary agents has evolved as an effective strategy to control the incidence of cancer. Epidemiological studies have demonstrated a positive correlation between increased consumption of vegetables, fruits, and beverages with reduced risk of cancer. The

mechanisms responsible for this chemopreventive effect still remain largely unknown but are likely related to the presence of phytochemicals associated with fruits and vegetables

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