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DAPHNE GENKWA SIEB. ET ZUCC. AS ANTICANCER OF ORAL SQUAMOUS CELL CARCINOMA : A SYSTEMATIC REVIEW

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ABSTRACT : Oral squamous cell carcinoma (OSCC) is the most common type of oral malignant neoplasm. It is weird cancer incidence due to hardly to prognosis. Natural resources have been optional treatment to any kind of diseases, including cancer. *Daphne genkwa* is herbal plant that have been used as chinese traditional medicine and studied for a long time ago as illness treatment. The present sudy was performed anticancer properties of *Daphne genkwa* to OSCC. The qualitative systematic review were obtained from Google Scholar, Medline, PubMed, Research Gate and Scopus. Data selection criteria are followed with keywords such as *Daphne genkwa*, herbal plant, phytochemistry, oral squamous cell carcinoma, chemoteraphy, and anticancer drug. *Daphne genkwa* had anticancer activities through multiple mechanism inhibition of cellular movement, cell cycle arrest, and blocking cell proliferation in oral cancer cells. *Daphne genkwa* have capability to developed as a promising anticancer source for OSCC. More extensive research are needed as clinical trials to determine which active compound that have best results as anticancer candidate.

Key words : *Daphne genkwa*, oral cancer, oral squamous cell carcinoma, medicine.

INTRODUCTION

Oral cancer is one the lethal disease and be top ten most frequent cancer mostly in developing countries. Scientist have identified different stage of cancer to develop, indicating that some gene mutation lead to abnormal cell proliferation and change cell function. Every year the prevalence of cancer is increasing and still affecting the health of human societies (Siegel *et al*, 2016; Hassanpour and Dehgani, 2017). Oral squamous cell carcinomas (OSCC) were reported that more than 90% found in the tongue, mouth, lips, gingiva and oropharynk. OSCC can occur in all areas of the body, however they are most common in the oral cavity and skin. OSCC exhibit 2-4% diagnosed malignancies and 9% distant metastasis annually in the United States. Upward trend of OSCC also spread in eastern and western europe (Massanao *et al*, 2006; Markopoulos, 2012; Abraham *et al*, 2019).

There are some related factor of OSCC. Gender is none factor of OSCC, there is no prognostic differences between male and female. However, age is related factor that seems controversial, some reseachers show no correlation, whereas others indicate OSCC worse in older patients (Chen *et al*, 2009). OSCC risk development to the primary tumor is higher for smoker and alcohol drinkers. The environment of societies are also most affect to this disease. The lower education and socioeconomic status seem highly have proper oral hygiene also difficult entering the medical care at least to check or control their oral condition. The survival rate of the OSCC patients may worsen if they have other diseases such as arrhythmias, heart failure, pulmonary, renal, and vascular (Markopoulos, 2012).

Nowdays, the standard to diagnose the oral diseases commonly using biopsy-histology. This method take a times to get the result, thus the patient have to wait for the treatment (Cervino *et al*, 2019). Chemotherapy drugs is a remarkable invention that have numerous benefits and also bad effects for the body. Therefore, the drug need to be modified or found another novel drug that not only to treat oral cancer but also be additional drug to delay the cancer growth. Herbal plant is smart alternative

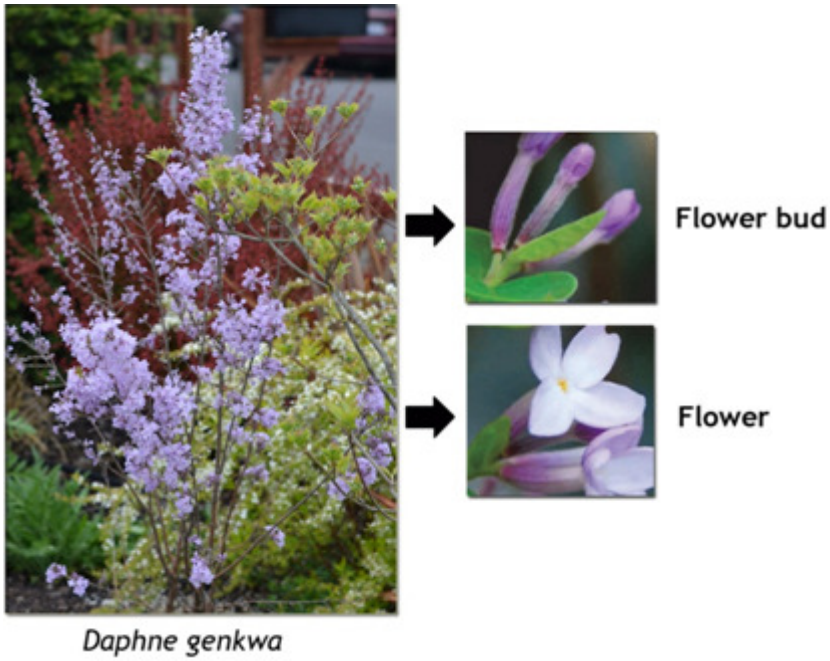

Fig. 1 : Plant of *Daphne genkwa* (Feng *et al*, 2018; Oliver, 2019).

to manage cancer development. It has many good results in research as an optional treatment for diseases. Today, it has been estimated that there are more than 300,000 herbal species, however about 10% used in community (Kartal, 2007; Fadholly *et al*, 2019; Huang *et al*, 2019). One of the best candidate to develop is compounds of *Daphne genkwa*. Previous studied had demonstarted that this plant exhibits anticancer effect, but still need more research to gain the best results. Hoewever, the study of *Daphne genkwa* for oral cancer is still limited. We systematically review to collect the literatures for investigating this issue.

Daphne genkwa overview

Daphne genkwa Sieb. et. Zucc. have local name yuan hua (Chinese), it belongs to Thymelaeceae family. *Daphne genkwa* is a medicinal plant widely distrubuted mostly distrubuted in Africa (tropical), Asia (sub-tropical) and Europe. Plants from the genus *Daphne* cover 95 species (Agnihotri *et al*, 2010; Juškoviæ *et al*, 2010). *Daphne genkwa* grow well in margin of paddy fields, hillsides, valleys, plains, limestone cliffs and on boulders. It is hermaphrodite and pollinated by bees, flies and lepidopetra. This plant is best in a deep rubbly well-drained soil and kept well altered in a dry growing season. It also have height to 1.5 m (5 ft), see Figure 1 (Li *et al*, 2013).

Ethnomedicinal and pharmacological used of *Daphne genkwa*

Daphne genkwa has been used as Chinese herbalism over 3,500 years. It is considered as one of the 50 fundamental herbs. The roots are used as anti-malaria, tonic, wound healing, and mushroom poisoning (Sovrliæ and Manojloviæ, 2016; Yang *et al*, 2017). The flower buds are a bitter acrid herb, used to control coughs. They are also used as an abortifacient and applied externally in frosbite treatment. The flowers are used as anti-cancerous, anti-inflammatory, anti-tussive, anti-viral, analgetic, expectorants, tranquilization, immunomodulatory agents and relief of the rheumatic sympoms in China and Korea as traditional medicine (Park *et al*, 2006; Lee *et al*, 2009).

Phytochemical of *Daphne genkwa*

Previous studies revealed that phytochemical of *Daphne genkwa* have pharmacologically active flavonoids, diterpen orthoesters, lignan, coumarin and sever other less common groups. A list of of secondary metabolites isoaled from *Daphne genkwa's* compounds are shown in Table 1.

The flower of *Daphne genkwa* contain flavonoid fraction such us luteolin, 7-O-methylluteolin, tiliroside, sitoserol, benzoic acid, apigenin, hydroxygenkwanin and genkwanin were reported effectively on arthritis mice without obvious advers effects (Zhang *et al*, 2014). Apigenin and luteolin form this plant also reported as antivirus for influenza and respiratory syndrome (SARS-CoV) by blocking the xanthine oxidase (Wu *et al*, 2010; Ryu *et al*, 2010). Daphne diterpene also reported can exhibit anti-HIV activties (Zhao *et al*, 2020). Dried flower of *Daphne genkwa* were also reported against enterovirus 71by inhibting viral gate without producing cytotoxic effects (Chang *et al*, 2012).

Table 1 : List of secondary metabolites isolated from *Daphne genkwa*.

Compound		Bioactivity	References
Coumarins			
1	Isodaphnoretin (monomeric)	NA	Zheng *et al*, 2005
2	Isodaphnoretin (dimeric)	NA	Zheng *et al*, 2005
3	Edgeworoside C (dimeric)	NA	Li *et al*, 2016
Flavonoids			
4	Apigenin	anti-carcinogenic	Funakoshi-Tago *et al*, 2011
5	Luteolin	anti-carcinogenic	Funakoshi-Tago *et al*, 2011
6	Velutin	NA	Park *et al*, 2006
7	Genkwanin	anti-carcinogenic	Li *et al*, 2017
8	Genkwanol B	anti-RSV activity	Huang *et al*, 2010
9	Daphnodorin B	anti-carcinogenic	Zheng *et al*, 2006
10	Hydroxygenkwanin	Anti-carcinogenic	Wang *et al*, 2013
Lignans			
11	Genkdapin (furofuran)	NA	Wang *et al*, 1990
Daphnane diterpens			
12	Yuanhuacine	anti-carcinogenic	Kang *et al*, 2016
13	Yuanhuadine	anti-carcinogenic	Wang *et al*, 1981
14	Yuanhuafine	NA	Wang *et al*, 1982
15	Yuanhuatine	anti-carcinogenic	Park *et al*, 2006
16	Yuanhuajine	NA	Zhang *et al*, 2006
17	Yuanhuagine	anti-carcinogenic	Zhang *et al*, 2006
18	Yuanhuahine	anti-carcinogenic	Hong *et al*, 2010
19	Yuanhualine	anti-carcinogenic	Hong *et al*, 2010
20	Yuanhuapine	NA	Sha *et al*, 1986
21	Genkwadaphin	anti-leukimia	Liang *et al*, 2010
22	Genkwanine-A to -K	anti-carcinogenic	Zhan *et al*, 2005
23	Genkwanine L (resiniferoid)	anti-carcinogenic	Zhan *et al*, 2005
24	Genkwanin-M (6-epoxy daphnanes)	NA	Zhan *et al*, 2005
25	Genkwanin-N (6-epoxy daphnanes)	anti-carcinogenic	Li *et al*, 2010

*NA: not applicable.

The flower buds were identified have ten compounds as octasane, doriacontane, beta-sitosterol, 4'7-dimethoxy-5-hydroxyflovone, aurantiamide acetate, genkwanin, luteolin, apigenin, 3'-hydroxygenkwanin and daphnoretin (Wang *et al*, 2009). Neogenkwanin I is the new daphnane-type isolated from flower buds that reported as antitumor (Hou *et al*, 2018). Genkwanin were also identified can inhibit inflammatory medium through MAPK/MPK/miR-101 pathway (Gao *et al*, 2014). They also against the production of nitric oxide (NO) that related in cell defence in RAW-264.7 and neurotransmission (Da-Yu-Li *et al*, 2014; Jiang *et al*, 2014).

Daphne genkwa's root also contain coumarin, diterpenoid, and flavonoid. Flavonoid in the root mainly comprised of daphnodorins-B, -G, - G-3"-methylether, -H, and -H-3"-methyleter. Six daphnodorins are responsible as inhibitory for metastasis of the tumor growth (Zheng *et al*, 2006).

Daphne genkwa for oral squamous cell carcinoma

Herbal products are supposed to managethe cancer cells with low toxicity and less side effects. Extracts of *Daphne genkwa* were reported as potent anti-proliferative activity *in vitro* against HT-29, B16, SW-480, Colo-205, HL-60, A-549, MCF-7, AGS, A-2058, K-293,MDA-MB-231, SMMC-7721, Huh-7, Ketr-3, P-388, MGC-803 and Hep-G2 cancer cells (Hong *et al*, 2010; Lil *et al*, 2013; Chen *et al*, 2014; Du *et al*, 2016; Wang *et al*, 2018). This plant also showed excellent results on some pharmacologic effects in mice that challenged with azoxymethane (AOM), omithine decarboxylase (ODC) through *in vivo* study (Kai *et al*, 2004). OSCC has an important local invasive capacity. The phenomena of invasive and metastatic are the section of cell migration, cell adhesion, rearrangement of cytoskeletal, basement membrane degradation and survival in the blood stream. Thus, a drug must be developed to stop that condition with making research. Some cancer cell lines of OSCC might be the first line to evaluate this plant as anticancer

for oral cancer. These cells are KON, Sa3, HSC-2 to 4, Ca9-22 and HO-1-u-1 (Koike *et al*, 2013).

Yuanhualine and yuanhuahine are two novel compounds of *Daphne genkwa* that related with daphnane diterpenoids yuanhuacine and yuanhuadine to inhibit activities on DNA topoisomerase I and cancer growth (Zhan *et al*, 2005; Zhang *et al*, 2006). Yuanhuadine was also related with EGFR, mTOR, or cMET signal production in cancer cells. Blocking those signal will decrease the risk of cancer growth (Hong *et al*, 2011). It is crucial phase to diagnose of peroral cancer lesion. Another study showed that yuanhualine, yuanhuahine and yuanhuagine showed the potent inhibitory against cancer growth. as. The growth inhibiton is relateable to cell arrest at G0/G1or G2/M phase. These phase correlated with checkpoint proteins including p21 and p53. When the p53 is inactivated, it could be first phase for oral cancer growth due to can not stop the cell growth with DNA damage. Cyclin-A, -B1, -E and phosphorylation of retinoblastoma (Rb), cMyc are a down-regulation of cyclin-dependent kinase (CDK). The mutations of Rb for oral cancer is rare. Normally, the mutation of Rb of being tumor form are more than 64% for premalignant lession and 25-69% for oral cancer. Thus, Rb could be the oral cavity biomarkers (Jo *et al*, 2012; Cerviono *et al*, 2019).

Flavonoids of *Daphne genkwa* can inhibit pro-inflamatory cytokine-induced chemokine expression. Flavonoid constitued the majority of the secondary metabolites. It posses antiproliferative correlated as antitumor through epithelium hyperproliferation inhibition, blocking the DNA synthesis and apoptosis promotion (Wang *et al*, 2018). Flavonoid of *Daphne genkwa* showed significant increase in life span, interleukin (IL)-1α, -1β, -6, granulocyte colony-macrophage and -stimulating factor production also reduce the tumor number in cancer cells *in vivo* and *in vitro* (Du *et al*, 2016). Literatures also showed that flavonoid as immunomodulatory for the immune defence that be the main key as antitumor agent of the host organism (Guo *et al*, 2015; Xu *et al*, 2015). *Daphne genkwa* activate the macrophages which produce cytokines, including IL-2 and IL-6. Cytokines are central regulators of immune defence that organize the immune responses in many tumor types and be a prognostic index for tumor growth in oral cases (Halloran, 2010; Lippitz, 2013).

Five major flavonoids in *Daphne genkwa* are luteolin, apigenin, hydroxygenkwanin, genkwanin and daphnodorin B (Fig. 2). Luteolin and apigenin effectively blocked TNFα, NF-κB through activation NF-κB p65 and GAL4DBD-p65/RelA, then distinct abnormal effect on the inflammatory responses in oral disruption (Orlichenko *et al*, 2010; Funakoshi-Tago *et al*, 2011). Daphnodorin B at doses of 40 and 80 mg/kg effectively maintained-protected the number of lympocytes proliferation, blocking the DNA synthesis, thus the cancer cells are not be able to do metastasis (Zheng *et al*, 2006).

Hydroxygenkwanin were found inducing the mitochondrial membrane potential (MPP) injury with cell arrest at S phase that initiated mainly by cyclin-dependent kinase (CDK)-2 which managed by abudance of p21 as a CDK inhibitors. The activation of caspase-3, -8, TNF-α, bid, bax were increased. However, bcl-xl protein decreased as imporntant sign in dysplastic oral lesions and in oral cancer (Wang *et al*, 2013). Additionaly, Hydrogenkwanin induced the miRNA expression, thus,

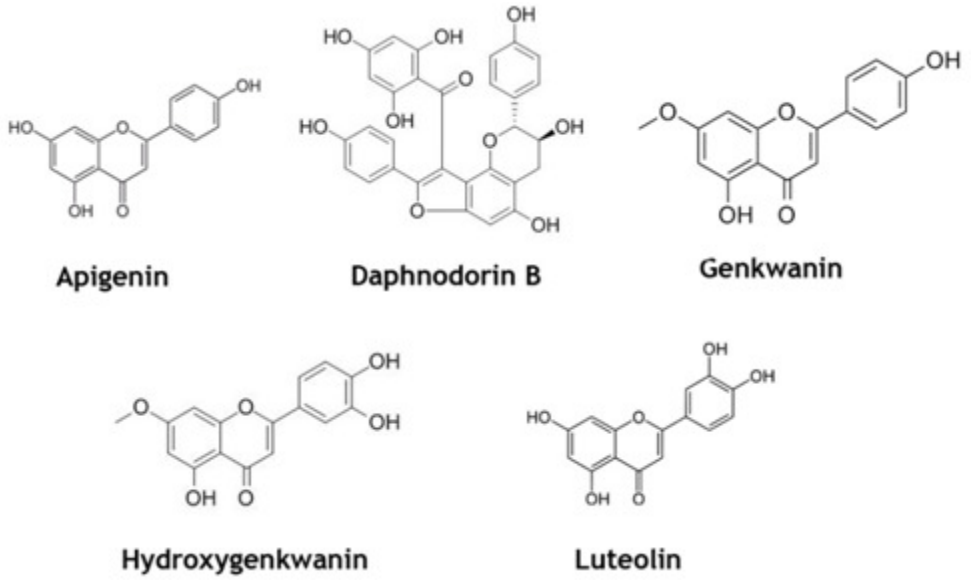

Fig. 2 : Some of phytochemical contents of *Daphne genkwa* (Jiang *et al*, 2014).

inhibit the transcription factor FOXM1 expression that are associated with epithelial-mesenchymal transition (EMT) genes such as vimetin, N-cadherin, twist and snail, then lead to suppress cancer growth and metastasis. miRNA level is prominent index as a prognosis on the basis of clinic pathological criteria of oral cancer patient (Gartel, 2017; Chou *et al*, 2019).

Genkwanin is a non-glycosylated flavonoid. It has been known as antitumor against some cancer cells. Genkwanin effectively blocked the tumor growth by reducing the inflammatory cytokine levels. The *in vivo* study demonstrated intervenous administration, genkwanin inhibited tumor growth rate of 62%. These results can also be applied for oral cancer (Androutsopoulos *et al*, 2009; Li *et al*, 2017).

Some of bioactive compound like genkwanin have insolubility and low bioavailability, to overcome this problem, nanosuspensions may become a solution. Nanosuspension is a delivery system of nanoscale drug that effective attetion to change the insoluble drug to be more soluble drug and their suitability problem administration routes for clinic application using precipitation combined with ultrasonication method (Han *et al*, 2014; Li *et al*, 2014; Fadholly *et al*, 2020). Stabilizer play important role in nanosuspension preparation. Some research showed that adding drug-stabilizer with ratio of 1:1, resulted diameter less than 200 nm with the increase drug-loading content and stability (Li *et al*, 2017).

CONCLUSION

Taken together, our literatures demonstrate that bioactive compouds of *Daphne genkwa* may be mainly attributed to develop as promising anticancer agent for targetting oral squamous cell carcinoma. To our knowledge, *Daphne genkwa* had anticancer activities through multiple mechanism blocking cell proliferation, cellular movement, and cell arrest in oral squamous cell carcinoma.

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