ANTI CERVIX CANCER AND ANTIOXIDANT ACTIVITY OF EDIBLE SEAWEEDS *Halimenia durvilae* OBTAINED FROM COASTAL AREA OF NORTH SULAWESI

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INTRODUCTION

Cervix cancer is the commonest cancer cause of death among women in developing countries.[1] Mortality due to cervical cancer is also an indicator of health inequities, as 86% of all deaths due to cervical cancer are in developing, low- and middle-income countries.

Based on the data of GLOBOCAN project in 2012, 235,000 death cases caused from cervical cancer from a total of 14,067,894 new cancer cases and 8,201,575 death cases were recorded worldwide (Internasional Agency for Research on Cancer (2015).

Various treatments have been made to cure cancer, including surgery, chemotherapy, radiotherapy, and targeting therapy. But, all of these procedures have side effects, such as vomiting, malaise, anemia and susceptibility with infection.

At present has been found some anti virus drug such as podophyllin or trichloroasetat acid, which used in USA and Europa. However the price of sintetic drug is too high and show side effect, therefore the people trigered to use traditional drug from natural product, that have been used for long time. (Syukur & Hernani, 2000)

Nowday, the use of natural product is a complementer alternatif to treatment breast cancer, cervix cancer and vagina cancer. In Indonesi there are 61.8 % patient of cervical cancer used natural from plant beside comercial drug (Radji et al., 2010).

Seaweed or macro algae contents bioactive compounds, such as phenols, fatty acid and dietary fiber that can prevent degenerative diseases (inflamation, diabetes, cardiovasculer, hypertency, cancer) (Sanger *et al*, 2018.

Halimenia durvilae is a red edible seaweed, it grows abundantly in Indonesia, especially in North Sulawesi, in South East Asia is cultivated used by human as food, it is usually served raw as salad. this seaweed has mot yet use for healthy, because there are not report its bioactive activity The main objective of the present study was to evaluate antioxidant activity and Anti-cancer cervix activity.

Most experimental data indicate that free radicals have a role in the initiation and promotion of cancer (Cross et al. 1987). Initiation and promotion of tumors involve changes in DNA either as a result of an inherited genetic anomaly or damage to the DNA strand.

In view of the association between DNA damage and carcinogenesis, it is likely that any agent capable of modifying DNA could be carcinogenic. Free radicals fall into this category.

Other than direct damage to DNA by free radicals, oxidative damage to lipids and to proteins such as DNA repair enzymes could also lead to DNA mutations

Type of Free radicals

superoxide anion (O^{-2}),

hydroxyl radical (•OH),

peroxyl radical (ROO•),

Alcoxil radical (RO•),

Lipid peroxil radical (LOO•),

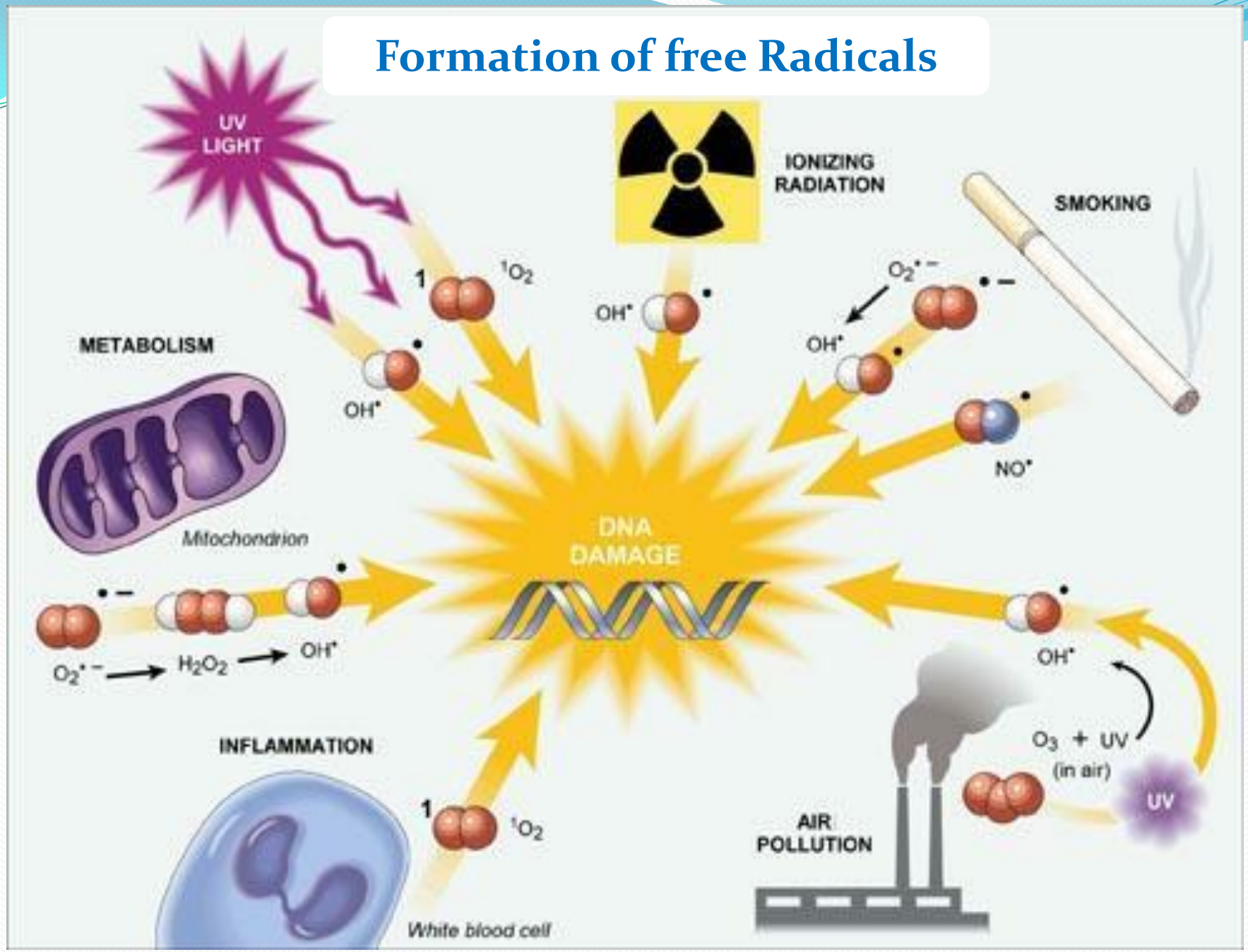
hydrogen peroxide, (HOOH)

nitric oxide radical (NO),

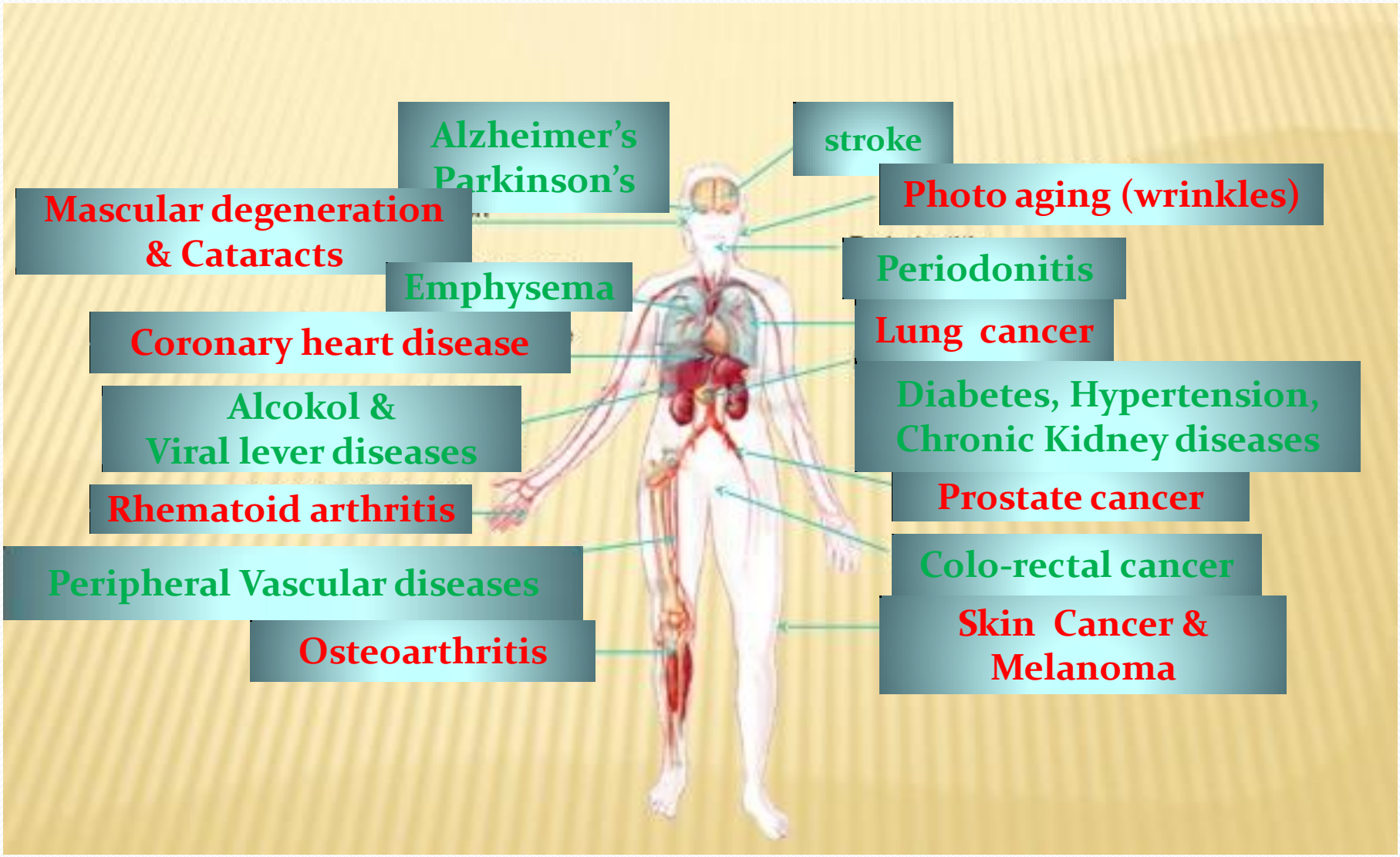
Singlet oxygen.

These molecules are unstable and highly reactive, and can damage cells by chain reactions, such as lipid per oxidation or formation of DNA adducts that could cause cancer-promoting mutations or cell death.

Formation of free Radicals



Free Radical diseases

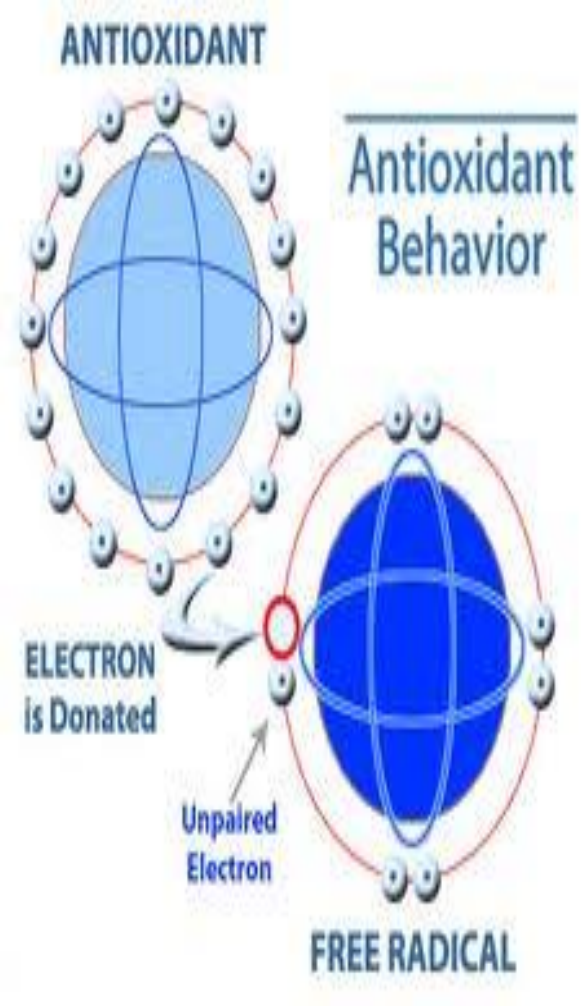


Antioxidant

Antioxidant is a molecule capable of inhibiting the oxidation of other molecules.

Oxidation is chemical reaction that transfers electron or hidrogen from a substance to an oxidation agent.

Oxidation reaction can produce free radicals. These radicals can start chain reaction



The function of antioxidant

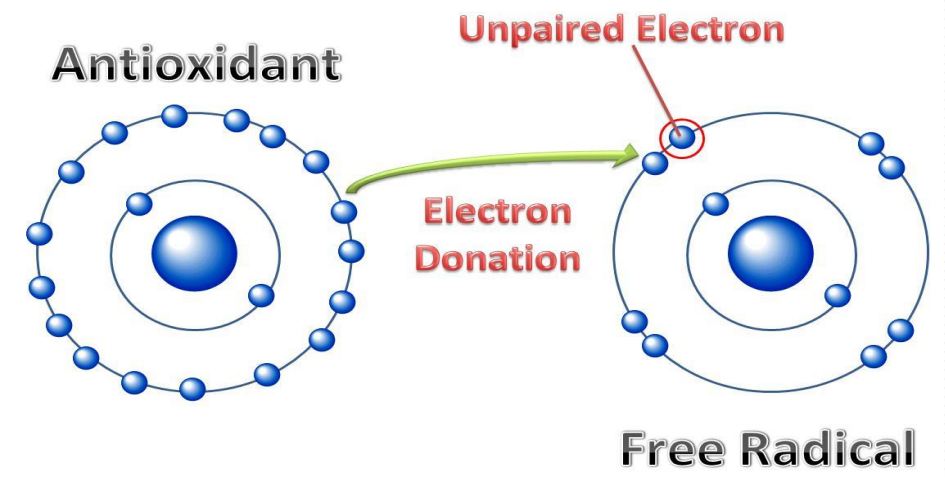
- Slow or prevent damage to body cells
- May improve immune function and lower risk for infection and cancer.

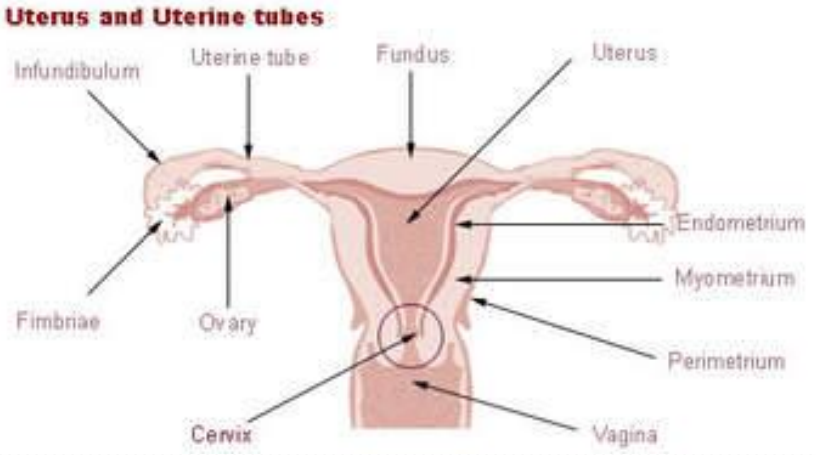
Example: Carotenoids

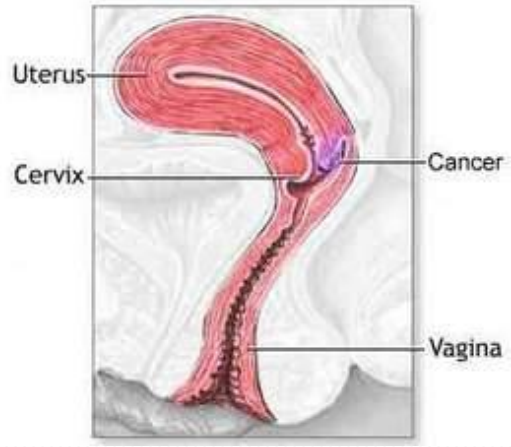
Vitamin C

Vitamin E

Found in colorful fruit/veggiess and grains.







The cervix located in the lower part of the uterus, the place where a baby grows during pregnancy. The virus spreads through sexual contact. Cervical cancer caused by HPH (Human Pappyloma virus). HPV comprise more than 200 types of infections, among which the high-risk HPV types 16 and 18 are the main cause and account for about 70% of cervical cancer. Most women's bodies are able to fight HPV infection. You're at higher risk if you smoke, have had many children, use birth control pills for a long time, or have HIV infection.

Normal cells become cancer cells through a wide range of genetic changes, and this process generates many different types of cancer . However, most cancer types share similar characteristics, and these must be studied to progress anticancer drug discovery and cancer treatment.

Hanahan and Weinberg identified six major targets in human tumors: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis

Marine compounds that play a role in some of the hallmarks described by Hanahan and Weinberg. Then, they have classified marine natural product as growth inhibitors and anti-tubulin agents, inductors of apoptosis and autophagy, and anti-angiogenic, anti-migration, anti-invasion and anti-metastatic agents. In addition, due to their relevance in signal transduction pathways, a supplementary family that includes inhibitors of proliferation and of mitogen-activated protein kinases (MAPKs) are also included.

Cytotoxycity test toward cancer is a general basic test for anticancer drug and chemopreventive compounds. One of method that used generally for in vitro cytotoxycity test is MTT method . This method is based on reduction reaction of MTT reagent (3-(4,5-dimethylthiazole-2-yl) 2,5- diphenyltetrazolium bromide) which catalyzed by dehydrogenase succinate enzyme in human cell.

MATERIALS AND METHODS

Material

Halimenia durvila was collected from Arakan Manado Indonesia in the period February 2015 respectively. The sample was thoroughly washed with seawater and fresh water to remove epiphytes and dirt particles. They delivery to laboratory and were stored at -20°C. Until further use.

Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), MTT (3-(4,5-dimethylthyazol-2-yl)-2,5 diphenyl tetra-zolium bromide), hella cell ((ATCC CC2 were purchased from Sigma Aldrich, All other solvent and chemicals were of analytical grade

Preparation of sample extract

Freeze sample extracted using methanol overnight for 3 times at room temperature, filtered with filter paper Whatman No. I and concentrated down at 40℃ by rotary evaporation. The extract obtained was decantation using ethyl acetate and fractionated contionouslly using hexane, chloroform and water, after that they were evaporated. Extract and fraction storage at -20℃ for further analysis.

Method of analysis

1. Antioxidant aktivity using of stable1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals assay.
2. Anti-cancer cervix using MTT (3-(4,5-dimethylthyazol-2-yl)-2,5 diphenyl tetra-zolium bromide) assay.

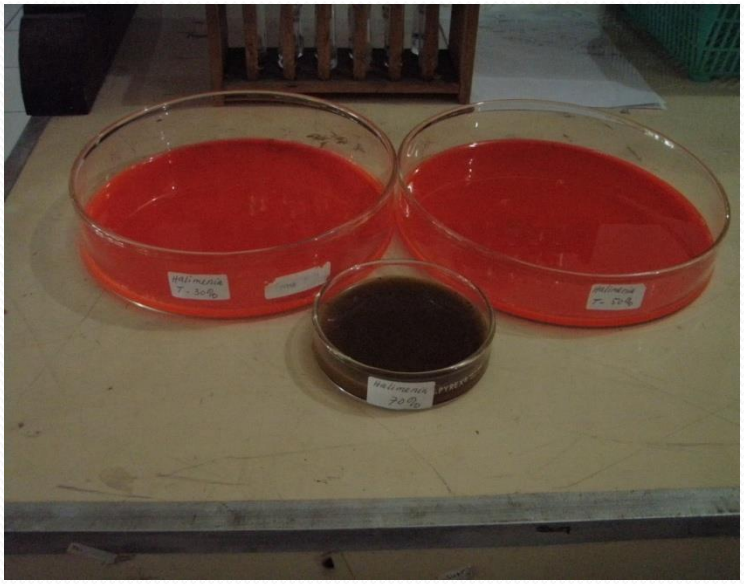
Statistical analysis

All experiments were conducted in triplicate (n=3).

The means of parameters anticencer and antioxidant actifity present as mean ± standart deviation. The data were analyzed by using statgraphicCenturion IX software.

RESULT & DISCUTION

- Extract of seaweed



1. Scavenging radical dpph

The result of analyzis shows that DPPH radical scavenger of water fraction was highest, followed with choloroform, metanol and hexane

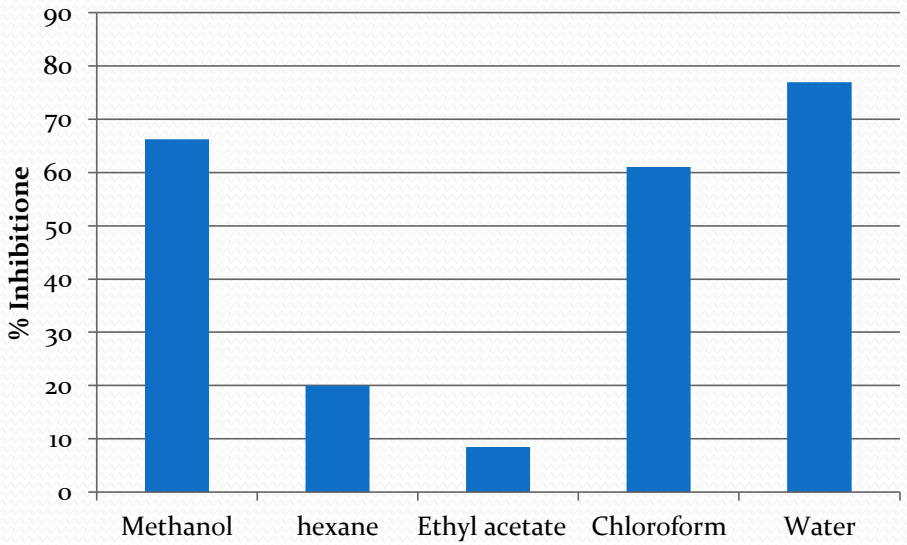
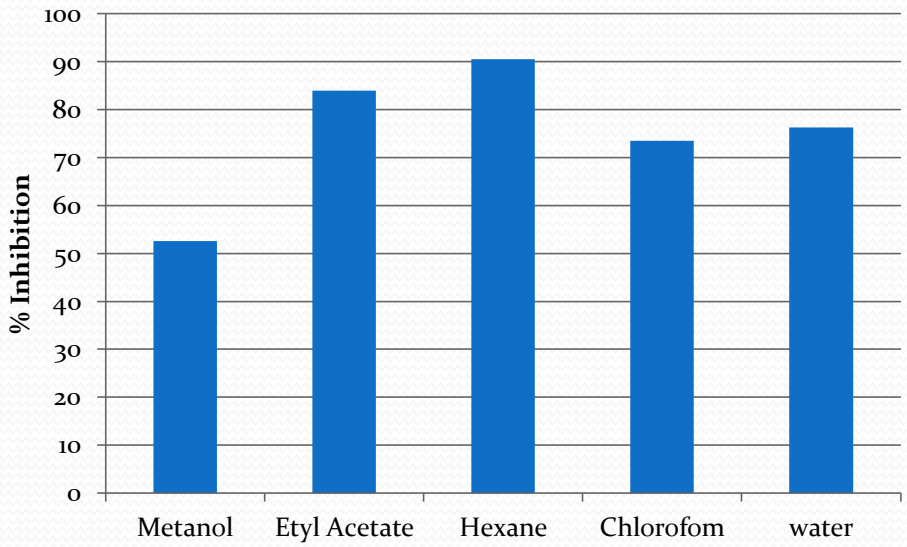


Fig.2. DPPH radical scavenging activity (%) of total methanolic extract and fractions obtained from H. durviae

. Anti-cancer servix activity

- The result shows that anti-cancer activity of hexane fraction was highest.



- **Fig.3. Anti-cancer cervix activity (% inhibition) of total methanolic extract and fractions obtained from H. durvilae**

The research respectively showed the highst cytotoxxyty to against HeLa cell wa the hexane fraction, with inhibition activity of 93.05 ± 5,4g % (125 µg/mL. The highst antioxidant activity against free radical was water fraction, IC 50 5.18±0.4 mg/mL

Edible seaweeds contain appreciable amounts of polyphenols which are effectives antioxidant and may have particular biology activity. For example, polyphenol-rich extracts and isolated phlorotannin compounds have been showed to inhibited proliferation of cancer cell and influence anti-inlammatory responses (Yuan *et al.*, 2005; Kim *et al.*, 2009).

Carragenan is polysaccharide sulfate from saccharide sulfat of D-galactose anf 3,6-anhydro-D-galactose extracted from red alga has been used in industry pharmacy, cosmetic and food. Many research showed Carragenan have activity to against few virus such as HIV, HSV and influenze virus. Carragenen especially L-carragenan are more potencial against HPV infection than heparin. Sulfat polysaccharide of red alga are effective inhibit pseudovirion HPV virus in IC_{50} 0,27 µg/mL

Caragenan with compound that have sama structure such as dextran can prepared as vaksin-peptida HPV vaxine (Buck et al., 2006)

Buck *et al* (2006) reported that the sulfated polysaccharide agar derived from red algae could also effectively block HPV pseudovirion infection with the IC50 value of 0.27 μg/mL. In addition, carrageenan and its structurally related compounds such as dextran can also serve as adjuvants for enhancing peptide-based HPV vaccine potency . Iota carrageenans possess good anti-HPV activities in vitro and in vivo, we suppose that the sulfated galactose structure and the optimal sulfate content are very important for anti-HPV actions of carrageenans.

CONCLUSION

In the present study can be concluded that H.durvilae has bioactive compound which have function as an anti-oxidant and anti-cervix cancer, so it can utilized as a source of natural antioxidant.

The sulfated polysaccharides derived from red algae especially carrageenans merit further investigation as novel anti-HPV agents in the future.

This study is useful to future research to isolate and identified bioactive compound that responsible for highest antioxidant or anti-cancer servix.

Thank you