

International Journal of ChemTech Research

CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555 Vol.12 No.06, pp 109-115, 2019

ChemTech

Effect of Different Drying Methods on Phytochemical Content of *Caulerpa lentillifera* from Kei Islands

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Abstract : : Seaweed, *Caulerpa* sp., is one of the natural materials that contain many kinds of bioactive compounds depending on species and habitat. The aim of this study was to know the effect of sun drying method on the phytochemical content of *C. lentillifera* from Kei Islands waters, Southeast Moluccas. The study consisted of several steps, *C. lentillifera* sample collection using sampling purposive method, direct sun drying and indirect wind drying methods, methanol extraction, and phytochemical test using Harborne method. Crude extract of *C. lentillifera* in indirect wind drying method was higher than that in direct sun drying. Alkaloid, terpenoid and steroid were found in *C. lentillifera* from Kei Islands on both drying methods. Saponin was only found in indirect wind drying methods. **Keywords** : bioactive compound, natural material, seaweed.

Introduction

Caulerpa sp is one type of green seaweed that grows in coastal waters, can be found throughout the year in the Kei Islands and is known to the community by the local name "lat"[1].Seaweed has important an economic potential for human used as comestible, main ingredient on health and industry. FAO data in 2000 showed that the use of seaweeds in pharmacy is still very low compared with its usage in food and farm industries, for example sheet carrageenan[2].Seaweeds contain specific chemical compounds as secondary metabolites to fight diseases and other organisms in their habitat that they can survive in extreme sea conditions. These chemical compounds are known as phytochemical compounds with pharmacological activities, such as anti-bacteria, anti-fungi, anti-virus, anti-protozoa, anti-inflammation, and etc.

Alfonsina Marthina Tapotubun et al / International Journal of ChemTech Research, 2019,12(6): 109-115.

DOI= <u>http://dx.doi.org/10.20902/IJCTR.2019.120614</u>

Phytochemical test is a qualitative test of bioactive compounds[3].Nevertheless, some bioactive compounds are unstable and possible to disappear as result of certain method application, such as high temperature drying. Many studies have shown bioactive compound potential of sea algae extracts from various places as raw material of anti-bacteria[4,5,6],anti-fungi, and anti-inflammation[7], antioxidant[8], and pharmaceutical products[9,10].

Chemical compositions of seaweed highly vary depending on species, habitats, and seasons[11]. So far, there is no information in relation with phytochemical compounds in *Caulerpa lentillifera* from Kei Moluccas water. This study aims to know the effect of drying methods on the phytochemical content of *C. lentillifera* from Kei Islands waters, Moluccas.

Material and Method

Material

Some materials used in this study were *C. lentillifera*, methanol (merck). $HgCl_2$ (merck), KI (merck), dietil eter (merck), Mg powder. The tools were analytical sales (Apel-PD-3000 UV), spectrophotometer (Boeca), and rotary evaporator (Buchi).

Method

The study consisted of three steps, which were *C.lentillifera* sample collection, preparation, sample drying and phytochemical test.

Preparation

C.lentillifera samples were collected from Letman Village, Kei Islands, Southeast Moluccas, at the geographic position of 5°45'1"S and 132°43'41"E, and washed in running water. This study employed two drying methods, direct sun drying and indirect wind drying.

Drying method [11]

Drying process used 2 kg of fresh *C.lentillifera* sample. Direct sun-light drying was done by spreading the seaweed on the floor in an area directly exposed to the sun-light. In indirect wind drying, the sample was spread on the bamboo self and left to dry.

Extraction[12]

Dried *C.lentillifera* sample was extracted through maceration in methanol solvent. Maceration process used shaker bath for 24 h and then, filtered through Whatman filter paper. The extract was evaporated using rotary evaporator at 40-50°C to allay methanol residue until the crude extract was obtained. Crude extract rendement was calculated using the following formula:

Crude extract rendement (%) =
$$\frac{\text{weight of crude extract (g)}}{\text{weight of powder (g)}} \times 100$$

Alkaloid test[12]

Alkaloid content was detected using Meyer reagent. It was done by dissolving 0.2 g HgCl_2 in 6 ml aquadest and 0.5 g KI in 1 ml aquadest. Both solutions were mixed up to be homogeneous. *C. lentillifera* sample was dissolved by chloride acid 0.1 N, then continued with 2 hour- maceration, added with 2-3 Meyer reagent, and observed the color change. Yellow color is indicated as presence of alkaloid content.

Trepenoid/steroid test[12]

The sample was shaken quickly with 2 ml of chloroform, and then added with aquadest. The solution was shaken again and left until 2 layers were formed. Chloroform layer was dropped on the plate drops and let it dry, then added with some anhydrous acetic acid (up to 10 drops) and 2 drops of concentrated sulphuric acid. Positive steroid compound was shown with color change to blue or green.

Phenolic test[12]

Some drops of water layer were added with ferric chloride solution in the flask. Positive phenolic content was indicated with violet color.

Flavonoid test[12]

Some drops of water layer were poured into a flask, and then added with concentrated chloride acid and magnesium powder. Presence of flavonoid was shown with red color.

Saponin test[12]

Somecrude extract was re-extracted with diethylether and the soluble fraction was separated. The insoluble residue was added with 5 ml of aquadest, and shaken. Presence of saponin was indicated with stable foam for 15 min.

Result and Discussion

Extraction yield

Extraction is one of the separation method of pulling out the active ingredient using organic reagent. The solvent used should be able to extract the desired substance without dissolving other substance. Compounds are soluble in certain solvent if they have similar polarity. Maceration is the most appropriate method. Several studies have suggested that the use of methanol solution to extract the active compound can produce more rendement than other solutions[13,6]. In general, the active compounds of methanol extract in this study was high enough, but the wind-drying method produced more active compounds than that of direct-sun drying method, because high temperature in direct-sun drying method could destroy some components(Table 1). Temperature of the direct-sun drying method was higher than indirect method causing fast drying process so that the evaporated water was more than indirect drying method at the same time for its lower temperature. Husni *et al.* (2014) reported content of evaporated free water would be greater in high temperature so that dried material production would be lowered too[14].

Sample Code	Sample Weight (g)	Extract Weight (g)	Extract Yield (%)
Direct	49.9803	6.5570	13.1191
Indirect	50.0121	9.0901	18.1758

Table 1. Rendement of active component of C. lentillifera extract using different drying method

Bioactive compound can be obtained through extraction, a transferring process of dissolved component into the solvent. Methanol selection as a solvent was based on the material polarity, in which *C.lentillifera* is polar and could transport the active compound better. Bioactive compounds are extracted easier using solvent of the same polarity[12]. Several studies showed that the use of methanol solvent could produce more rendement than that using other solutions[6].Methanol solvent produced the highest number of bioactive compound extracts from 4 species of seaweeds, *C.racemosa, E.cotoni, Gracilaria* sp and*Sargasum* sp.[5]. Polar solvent could produce more *Caulerpa* sp extract than semi polar solvent (n-hexane) and non-polar solvent (chloroform)[12]. Methanol (methyl alcohol) is the most simple alcoholic compound, evaporable, flammable, 78.4°C boiling point and 114.3°C melting point. Methanol is universal solvent with polar cluster (-OH) and nonpolar cluster (-CH3) that could bind to other polar as well as non-polar compounds. Biochemical isolation from plants can be directed to certain dominant compound by choosing the paper solvent. Polar solvents tend to dissolve polar solutes and otherwise, non-polar solvents tend to dissolve non polar solutes[12]. Methanol is polar organic solvent naturally produced by microbial fermentation or anaerobic metabolism[15].

Phytochemical of Caulerpa lentillifera

Phytochemical are a group of secondary metabolites in organic materials. The phytochemical test was based on the color difference that occurs when the sample is reacted with a particular reagent suitable for determination of bioactive compounds in the sample. The compounds of the dry *C. lentillifera* found in phytochemical test with different drying methods are presented in Table 2.

Test	Dry C. lentillifera		Discoloration/Foam	
	Direct	Indirect	Direct	Indirect
Alkaloid	+	+	Yellow	Yellow
Terpenoid	+	+	Red	Red
Steroid	+	+	Blue	Blue
Flavonoid	+	+	Red	Red
Phenols	-	-	Yellow	Yellow
Saponin	-	+	No foams	Foams

Table 2.Phytochemical tests of dry C. lentillifera t with different drying methods

Alkaloid

Alkaloid is one of the most common phytochemical content found in seaweeds. The principle of alkaloid analytical method is precipitation through ligand exchange. Nitrogen atom that has free electron pair in alkaloid could replace the ion in the reagent. Positive alkaloid content is shown by yellow precipitate after reaction with Dragendorff reagent.

This study showed that direct sun drying method and indirect wind drying method did not allay the alkaloids, in *C. lentillifera* (Tabel 2). The alkaloid is alkalineand can be easily decomposed by heat and oxygen[15]. It suggested that alkaloid content in *C.lentillifera* could be high so that high temperature in direct sun drying does not allay this bioactive compound.

Alkaloid was detected in *Caulerpa* sp, *Eucheuma* sp, *Gracillaria* sp and *Sargassum* sp. extracted by methanol solvent. In n-hexane extraction, alkaloid was only found in *Caulerpa* sp and *Gracillaria* sp[12].

Biological activity of alkaloids results from the presence of nitrogen-containing base. In this situation, when it contacts with bacteria, it will react with amino acid and DNA of the bacteria that are major building component and center of various cellular activity[10]. Cell wall and most of bacterial nucleus are acidic because of comprising amino acids. Chemically, base compounds will react with acid compounds. Thus, amino acids of the bacteria will react with bases of the alkaloid resulting in structural changes in amino acid of the bacteria. Those changes will modify the genetic balance of DNA acid and damage the DNA of the bacteria. Destruction of DNA nucleus will trigger nucleus lysis and causethe bacterial cells havemetabolism inability so that the cell will also turn into lysis.

Many studies have shown that alkaloids were potential to be antibacterial and analgesic drugs[16]. Alkaloids can irritate the peptidoglycan building components, so that the cell wall will be improperly built and then lead to cell apoptosis[17].

Terpenoid

This study found terpenoids in *C. lentillifera* samples processed with sun drying method and wind drying method (Table 2). Temperature increase in sun drying method did not eliminate the terpenoids. This result probably indicates high terpenoids contained in *C. lentillifera*. Terpenoids are found in *Caulerpa* sp methanol extract but not found in *Eucheuma* sp, *Gracilaria* sp and *Sargassum* sp [6].

Green algae produce terpenoids and aromatic compounds as anti-inflammation, anti-microbial, antivirus, anti-mutagen and insecticidal agents[18]. Terpenoids are lipid-soluble compounds. This feature makes terpenoids able to penetrate the cell wall of both gram-positive and gram negative bacteria[19]. Steroids/triterpeoids inhibit bacterial growth through protein synthesis inhibition and cause changes in cell structure itself. Terpenoids play important role as anti-bacteria reacted with trans-membrane protein of the outer cell wall membrane of the bacteria and form a strong polymer bind that cause porin damage. Many terpenoids of the natural materials have potentials as toxic compounds[20].

Steroid

Phytochemical content of steroid was found in both dried *C. lentillifera* samples (Table 2). Temperature increase in direct sun drying method did not eliminate the steroids, and it could reflect that *C.Lentillifera* contains high number of steroid. These results are relatively similar to previous studies on *Caulerpa* sp from Tual coastal waters [21]. Steroids are the most common group of chemical compounds found in *Caulerpa* sp, *Eucheuma* sp, *Gracilaria* sp, and *Sargassum* sp using three types of solvents [6]. Steroids have potential as antibacterial and antifungal agents by cell membrane destruction mechanism, so that inhibits the growth [22]. Steroids delay bacterial growth process by protein synthesis inhibition and resulting changes on cell structure itself.

Flavonoid

Drying methods generally influence material content, but this study found that flavonoids were not influenced by direct sun drying method, nor indirect wind drying method. Flavonoids are able to inhibit bacterial growth under 2 different mechanisms: First, flavonoids trigger damage on cell wall permeability, mysosome and lysosome of bacteria as the result of flavonoid-DNA interaction[23]. Second, hydroxyl group in flavonoids causes some changes in organic component and nutrition transport process, then results in toxic effect on bacteria[6,22].

Flavonoids can create a bond with cell protein of bacteria by hydrogen binding. Proteins in cell wall and cytoplasm of bacteria become unstable after flavonoid-hydrogen binding, so that the proteins may lose its biological activity. Therefore, permeability of the cell will be disturbed and causing cell death by lysis process[12]. Flavonoids are disposed to bind with bacteria proteins so that inhibits the activity of enzyme, which irritates bacteria metabolism[24].

Flavonoids are found in plants as glycosides and a potential phenolic with antioxidant effect. The natural antioxidant of plant-originated flavonoids is beneficial to prevent cancer, protect cell structure, increase vitamin C effectiveness, prevent osteoporosis and play important role as anti-inflammatory and antibiotic agents[16]. Flavonoids in food are predictively important due to its strong antioxidant effect of phenolic compounds.

Qi *et al.*, (2008) reported that flavonoid berperan aktif sebagai antifouling[25].Flavonoids are also lipophilic compounds that will destruct the membrane of microbes. Flavonoids contain an acid phenolic compound known as carbonic acid[16]. Phenols are able to causing protein denaturation and destruct the cell membrane. Acid environment by phenol can influence bacterial growth process[24].

Phenolic compounds

The present study showed that phenolic compounds were not found in *C. Lentillifera* extracts, using either direct sun drying method or indirect wind drying (Table 2). This indicates that phenols do not occur in *C. lentillifera* from Kei Islands or exist only in small numbers so that long drying period could totally eliminate their presence. Temperature and duration of drying process can eliminate some phenols[26].Phenolic compounds could be totally released in 3 days from direct sunlight drying and 7 days from wind drying method. Temperature and long period of drying process could some phenols and in dry condition, all components in the cell, such as membrane and organelles, fuse so that phenol extraction becomes more difficult.

Phenol is also semi-polar, so that can be easily extracted by semi-polar solvent too. Polar compounds are more soluble in polar solvents, and non-polar compounds are more soluble in non-polar solvents. The polarity depends on dielectric constant, which is equal to polarity of the solvent[13]. Dielectric constant for some solvents are 33.60 to methanol, 6.02 for ethyl acetate, 1.89 for hexane, 24.30 for ethanol and 80.40 for water.

Increased drying temperature could lead to degradation of most phenolic compounds. Phenol is sensitive, unstable and degradable compound, which thermal, oxygen and light are the main degradators[26]. Different drying methods influenced the total number of phenolic inseaweed. Phenols contain an acid phenolic compound known as carbonic acid. Phenols are able to causing protein denaturation and destruct the cell membrane. Acid environment by phenolic can influence bacterial growth process[26,22].

Saponin

Saponin is one of antibacterial agent. Saponin can destruct the cytoplasm membrane of the bacteria and kill them. It can also increase permeability of the membrane and leadsto cell lysis [20]. The presence of saponin is indicated with stable foams production. Drying method influenced the saponin of *C. lentillifera* extract, and only the use of wind drying method canproduce foam. Direct sun drying method could eliminate the saponin in sample (Table 2). These suggested that *C. lentillifera* contains small amount of saponin and could be reduced by the drying method.

Compounds with polar and non-polar complex have an active surface, so that saponin can produce micelles when it was shaken with water. The micelles contain the polar complex outside and non-polar complex inside. This structure makes the micelles look like foams[12]. Saponin is one of antibacterial agent. Saponin fights against bacteria by destructing the cytoplasm membrane and killsthem.

Conclusion

Alkaloids, terpenoids, steroids and flavonoids were found in *Caulerpa lentillifera* from Kei Islands on both drying methods. Saponin was only found in sample using indirect wind drying method. Phenols was not found in *Caulerpa lentillifera*.

Acknowledgements

Thanks to a government of Republic of Indonesia through the Ministry of Research, Technology and Higher Edications. Directorate of Research and Community Services on research of Produk Terapan funds for the implementation of this study on research contract number: 236/UN13.3/LTLPPM/2019

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