Total Phenols, FlavonoidsandAntioxidantActivityofMethanolicandEthylAcetateExtracts of*Caulerparacemosa*Plants Grown in Usaha Jaya Village, Raja Ampat Regency, Southwest Papua Province

ABSTRACT

*Caulerparacemosa*is a speciesofgreenseaweedthatiswidelydistributed in almostalltropicalseas in theworld. Thosegreenseaweedcontainactivecompoundsthathavepotential as antioxidants. Differences in phenolicandflavonoidscontent as anantioxidantcanbeinfluencedbypost-takehandlingandsolventpolarity. The researchaimsto study thephenolicandflavonoidscontent in methanol (polar) andethylacetate (semi polar) solvents as well as theantioxidantactivityusing ABTS (*2,2-azinobis-3-Ethylbenzoathiazoline-6-sulfonic acid*), DPPH (*1,1-diphenyl-2-picrylhydrazyl*), and H_2O_2 (*Hydrogenperoxide*) methods. The highestextractyieldwasfound in themethanolextractwhichamountedto 6.12%, whiletheethylacetateamountedto 1.80%. The phenoliccontentofthemethanolextractwas 40.18 ± 3.06 mgGAE/g, whiletheethylacetateextract had thelowest total phenolcontentof 17.22 ± 1.37mgGAE/g. The total flavonoidscontentofthemethanolextractwas 84.42 ± 10.20 mgQE/ghigherthantheethylacetateextractwhichwas 78.61 ± 5.31 mgQE/g. Antioxidantactivityof ABTS methodofmethanolextractobtained IC50 valueof 134.64 ± 19.90 µg/mLwithmoderatecategorylowerthanthatofethylacetateextractwhichwas 113.50 ± 19.69 µg/mLalsowithmoderatecategory. Antioxidantactivityof DPPH method in methanolextractobtained IC50 reached 96.08 ± 0.45 µg/mLwithstrongantioxidantcategory, while IC50 in ethylacetateextractreached 167.72 ± 1.08 µg/mLwithweakcategory. The antioxidantactivityof H_2O_2 methodisclassified as moderateactivity in bothsolventsas inmethanolextractitwas 110.79 ± 2.52 µg/mLandin ethylacetateextractwas 123.67 ± 2.08 µg/mL.

Keywords: Antioxidant, ABTS, *Caulerparacemosa*, DPPH,H_2O_2, total phenoliccontent, total flavonoidscontent

1. INTRODUCTION

Indonesia isanarchipelagowith 75% ofitswaters. Indonesianwatershaveverydiversebiologicalresources, especiallymacroalgae[22]. Macroalgae are potentialbiologicalresources in intertidal coastalareasand are found in almostallpartsof Indonesian waters. Macroalgaegermplasmresourcesspread in Indonesianwatersamountto 6,42% ofthe total worldmacroalgaebiodiversity[34]. Sea grape (*Caulerpa*) isonetypeofgreenalgae (*Chlorophyceae*) that has ediblepropertiessothatitcanbeutilizedbyhumansfordirectconsumptionorprocessedintofoodand non-foodproducts[22].

*Caulerpa*containsconstituents as a sourceof natural antioxidants[8]. Research [11]revealedthatantioxidant-formingbioactivecompounds in greenseagrapesconsistofflavonoids, terpenoids, alkaloidsandphenols. The samethingwasalsorevealedby[10; 12; 41]. *Caulerpasp*.containssecondarymetabolitecompoundsincludingphenols, flavonoidsandsaponinsthatfunction as antioxidants. Thesebioactivecompounds are

formedthroughseveralbiosynthesispathwayswithchangesfromprimarymetabolitestointermediatemetabolites, thentosecondarymetabolites[3].

Raja Ampat Regency isoneoftheregions in Southwest Papua Provincethatisknownforitsabundantbiologicalresources, especially*Caulerparacemosa* were foundaroundthecoastalareasof Raja Ampat Regency and has beenconsumedbycoastalcommunities as a substituteforvegetablesand has become a daily diet. However, theanalysisofchemicalcomposition, total phenoliccompounds, andantioxidantactivityof*Caulerparacemosa*found in Raja Ampat waters has not beenanalyzed. The purposeofthis study wastodeterminethe total phenolicandflavonoidscontent in *Caulerparacemosa*extractswithmethanol (polar) andethylacetate (semipolar) solventsandantioxidantactivityusing ABTS, DPPH andH_2O_2 methodsobtainedfromthewatersof Usaha Jaya Village, Raja Ampat Regency, Southwest Papua Province.

2. MATERIAL AND METHODS

2.1. MATERIAL

SampleCollection: Sea grape (*Caulerparacemosa*) samples were obtainedfrom Usaha Jaya Village, EastMisoolDistrict, Raja Ampat Regency, Papua Province (1°59'16"S;130°24'59"E) onJanuary 2024. The samples were washedusingfreshwatertoremoveimpuritiesandthendriedatroomtemperaturebetween 28-32°C for 2-3 days. The driedsamples were putintoplasticsealsandpackedusingsterofoamboxesandthensenttothelaboratoryforfurther testing.

Material andInstrumentation : The materialsused in thetestofthe total phenoliccontent (TPC), total flavonoidscontent (TFC), andantioxidantactivityassay are; methanol 96%, ethylacetat 96%, ethanol 96%, Na_2CO_3, reagentFollin-Ciocalteu, gallicacid, reagentquercetin, aceticacid, $AlCl_3$ 4%, distilledwater, ascorbicacid, reagent DPPH, reagent ABTS, kalium persulfate, reagent H_2O_2, phosphatebuffer, and filter papers.The toolsused in thetest are; pestleand mortar, grinder, orbital shaker, rotary evaporator (Buchiirotavapor r-100), centrifuge, spectrophotometryuv-vissinglebeam (Shimadzu 1280), micropipette, glassware, funnels.

2.2. METHODS

- **SamplePreparationandExtraction**

The driedsamplesof*Caulerparacemosa* were cut intosmallpiecesandcrushedusing a grinder until they were powdered, thesamplesthat were stilllarge were againmashedusing a mortar andpestle until thesamplesbecamefinepowder (simplisia).A total of 25 g of simplisia wasdissolvedwitheachsolvent (methanolandethylacetate) intoerlenmeyer in a ratioof 1:10 thenplacedonan orbital shakerat 160 rpmfor 72 hours. After that, thesamplewasfilteredusing filter papertoproducesupernatant. The supernatantobtainedwascentrifugedfor 10

minutesandthentransferredinto a tube andevaporatedusing a rotary evaporator (buchiirotavapor r-100) with a bathtemperatureof 40-50℃ at 140 mbartoproduce a thickextract. The extractyieldwascalculatedbythe formula [4];

$$\text{Yield} = \frac{final\ product\ weight\ (g)}{Initial\ weight\ of\ raw\ material\ (g)} \times 100\%$$

- **Total PhenolicContent (TPC)**

A total of 50 mgofgallicacidwasdissolvedwith 50 mLof 96% ethanolinto a darkvialbottleandhomogenized. gallicacid as a standardsample,with a concentrationof 1000 ppm wasdilutedinto 20, 40, 60, 80, 100 ppm concentrationseries. Eachconcentrationwastaken as much as 1 mLplacedinto a test tube added 4% FollinCiocalteureagent as much as 0.4 mLhomogenizedandincubatedfor 4-8 minutesthenadded 5% Na_2CO_3 as much as 4 mLhomogenizedanddilutedto 10 mLusingdistilledwater. The absorbanceofthesamplewasmeasured in spectrophotmetrywith a wavelengthof 725 nm.

Eachextractsamplemeasured as much as 30 mgwasdissolvedusing 96% ethanol as much as 30 mLsothat a stocksolutionof 1000 ppm wasobtained. The determinationwasdone as a previouslydescribedwithgallicacid. Total phenoliccontentwascalculatedby making a calibrationcurveoftherelationshipbetweentheabsorbanceofgallicacidandtheabsorbanceoftheextractsamplewiththeformula [1,37].

$$\text{Total phenol GAE} = \text{c}.\frac{V}{m}$$

- **Total FlavonoidsContent (TFC)**

Quercetinstandardsolutionwasmeasured as 50 mganddissolvedusing 96% ethanol as much as 50 mLtoobtain 1000 ppm stocksolution. Dilutions were doneat 20, 40, 60, 80, 100 ppm. Eachconcentrationwastaken 1 mLandadded 1 mLof 4% $AlCl_3$and 1 mLof 5% aceticacidwashomogenized, thendilutedto 10 mLusingdistilledwaterandincubatedfor 1 hour. The absorbanceofthesamplewasmeasuredat 420 nmwavelengthusinguv-visspectrophotometry.

Eachextractsamplewasmeasured as much as 30 mganddissolvedwith 96% ethanol as much as 30 mLandhomogenized. 1 mLofthestocksolutionwastakenandthenthesamesteps were repeated as mentionedbeforewithQuercetinstandardsolution. Total flavonoidscontentwascalculatedbasedonquercetincalibrationcurve[24]withthe formula;

$$\text{Total lavonoid} = \frac{C \times V \times Fp}{m} \times 100\%$$

- **AntioxidantActivity**

ABTS (2,2-azinobis-3-Ethylbenzoathiazoline-6-sulfonic acid) assay

Testing theantioxidantactivityofthe ABTS methodbeginswiththepreparationof ABTS stocksolution. ABTS solutionwasmeasured 18 mganddissolvedusingdistilledwater as much as 5 mL, thenpotassiumpersulfatesolutionwasmeasured 3 mganddissolvedusingdistilledwater as much as 5 mL. ABTS andpotassiumpersulfatethathavebeendissolved are putinto a

measuringflaskandthe volume isadjustedto 25 mLusingethanol, thesolutionisthenincubatedfor 12-16 hoursatroomtemperature 22-24℃ [33].

Samplesextractof*Caulerparacemosa* 1000 ppm were taken as much as 100 µL, 200 µL, 300 µL, 400 µL and 500 µL thenadded 1 mLof ABTS solutionandthensufficient volume upto 5 mLusingethanolso as toobtain a solutionwith a concentrationof 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. Furthermore, itwashomogenizedandincubatedfor 15 minutesandthenmeasuredtheabsorptionat a wavelengthof 750 nm. The % inhibitionvaluewascalculatedusingthe formula:

$$\%\text{Inhibisi} = \frac{Abs.control\ of\ abts - abs\ of\ sample}{Abs.of\ control} \times 100\%$$

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The antioxidantactivitytestof*Caulerparacemosa*extractsampleswith DPPH methodreferso[17]which has beenmodified. Eachconcentrationseriesofextractsamples (20, 40, 60, 80, 100 ppm) wastaken as much as 3.5 mLandplacedinto a test tube thenadded 1.5 mLof 4% DPPH, thesolutionwashomogenizedandincubatedfor 30 minutesatroomtemperature (37°C) underdarkconditions.

Absorbancemeasurementwasperformedbyuv-visspectrophotometrywith a wavelengthof 517 nm. The controlusedwas 3.5 mLofmethanoland 1.5 mLof DPPH. The % inhibitionvaluewascalculatedwiththeequation[11].

$$\%\text{inhibition} = \frac{Abs\ of\ blank - abs\ of\ sample}{abs\ of\ blank} \times 100\%$$

H_2O_2 (Hydrogenperoxide) assay

Hydrogenperoxidescavengingactivityistheabilityof a compoundtoscavengeorremovehydrogenperoxide. Testing theantioxidantscavengingactivityof theH_2O_2 methodreferstotheresearchof[16]which has beenmodified. Extractsampleswithvariousconcentrationvariations were taken as much as 100 µL and 600 µL of 2 mMhydrogenperoxidewasaddedtothetest tube andthenthe volume wassufficientusingphosphatebuffer (pH 7.4) as much as 4 mLandthenhomogenized. The samplewasincubatedfor 10 minutesandtheabsorbancewasmeasuredat a wavelengthof 230 nm. The percentageofH_2O_2 scavengingactivitywascalculatedbasedontheequationbelow:

$$\%Inhibisi = \frac{Abs\ of\ control - abs\ of\ sample}{Abs\ control} \times 100\%$$

2.3. StatisticalAnalysis

The statisticalanalysisusedincludessimple linear regressionanalysisusingexceltools. Linear regressionis a commonlyusedanalysistodeterminetheformofrelationshipbetweentwoormorevariables.The coefficientofdeterminationisused in knowinghowmuchthevalueofthedependent /

dependentvariable (Y) ontheindependent / freevariable (X), thegreaterthe R^2value, thebettertheindependentvariablepredictsthedependentvariable. The valueof R^2 has anincreasinglylargerangebetween 0 and 1 [31].The equationusedis[43]:

$$Y = \alpha + bx + e$$

DescriptionY : antioxidantactivity, X : total phenolic/total flavonoids, αandb:regressioncoefficient, ***e***: standarderror/standarddeviation.

3. RESULTS AND DISCUSSION

Yieldextractof*Caulerparacemosa*

Dataofextractyield, total phenolsand total flavonoidsof*Caulerparacemosa*withmethanolandethylacetatesolvents are presented in Table 1.

Table 1. Extractyieldandphytochemicalcompoundcontent

Solvent	Yield (%)	TPC ± SD (mgGAE/g)	TFC ± SD (mgQE/g)
Methanol	6.12	40.18 ± 3.06	84.42 ± 10.20
Ethylacetate	1.80	17.22 ± 1.37	78.61 ± 5.31

Note: TPC (Total PhenolicContent), TFC (Total FlavonoidsContent), SD(StandartDeviation)

Table 1 showsthatdifferenttypesofsolvents in theextractionprocessaffecttheamountofextractproduced. Methanolsolvent has thehighestextractyield (6.12%) thanethylacetatesolvent (1.80) in accordancewiththeresearchof[11]methanolsolvent (polar) from*Caulerpalentilifera*extract has thehighestaverageextractyieldof 0.42% ofethylacetatesolventextract (semi-polar) and n-hexane (nonpolar) respectively, whichis 0.39% and 0.36%. Accordingto[18]themethanolextractof*Caulerparaemosa*istheextractwiththehighestyieldof 4.25% whileethylacetateis 2.08%.Basedonresearchby[17]saidthatthemethanolextractof*Caulerpafiliformis* had extractyieldsof 8.32% and 15.72%. Basedontheseresults, itshowsthat*Caulerparacemosa*contains polar and semi-polar compoundsthatcandissolvewell in methanolsolvents.

Extractionaimstoobtainoptimalbioactivecompoundsfrom a materials. The extractionprocessisstronglyinfluencedbyseveralconditionssuch as: extractionmethod, temperature, time, phytochemicalcompositionandsolventused. If theconditions in theextractionprocess are thesame, thenthesolventisanimportant parameter in theisolationofactivecompounds[39]. The amountofactivecompoundsthatcanbeextractedisinfluencedbythepolarityofthesolvent. Methanol has a fairlywidepolarityrangesothatthenumberofactivecompoundsthatcanbeextractedismore, both polar, semi-polar to non-polar compounds.

Total PhenolicContent (TPC)

The analysisof total phenoliccontentofmethanolandethylacetateextractsshowedphenoliccontent in methanolextract 40.18 ± 3.06 mgGAE/g andethylacetateextract 17.22 ± 1.37 mgGAE/g. The methanolextractof*Caulerparacemosa* has higher total phenoliccontentthantheethylacetateextract.

Table 1 showsthatthe total phenoliccontent in this study ishigherwhencomparedtotheresultsofresearchby[17]showingthatthemethanolextractof*Caulerpafiliformis* has a total phenoliccontentof 39.31 mgGAE/g fromSechura Bay and 18.78 mgGAE/g fromParacas Bay. However, theresultsofTable 1 are stilllowerwhencomparedtotheresearchof[11]wherethemethanolextractof*Caulerpalentilifera* has a higherphenoliccontent (154.65 mgGAE/g) thantheethylacetateextract (141.50 mgGAE/g). Theseresultsindicatethatdifferences in thetypeofsolvent in theextractionprocesswillaffectthe total amountofphenolicsproduced.

Accordingto[7]mostphenoliccompounds are polar. The resultsofresearchby[8]showthatthere are differences in the total phenolcontentproducedby*Caulerparacemosa*extractsfromdifferentsolvents, namelymethanolextract 66.61 mgGAE/g andchloroformextract 123.91 mgGAE/g. [29]statedthatthepolyphenolcontentofseaweed has variationsfromspeciestype, harvestage, season, andgeographicallocation. The processofextractionanddryingofsamplescanaffectthe total amountofseaweedphenols. The useofsolvents in theextractionprocessaffects total phenols[20]. The dryingprocessaffectsthe total phenolicsofseaweed[5;19]. Otherresearchersalsosaidthatdifferences in total phenolics in seaweed are causedbyseveralfactors, namely: seaweedtype, geographical, seasonal, physiological, andenvironmentalconditionsthatvary[15,6].

Total FlavonoidsContent (TFC)

Table 1 showsthe total flavonoidscontentofmethanolandethylacetateextractsof*Caulerparacemosa*fromthewatersof Usaha Jaya Village has a highervaluethan total phenolic. The total flavonoidscontentof*Caulerparacemosa*ofmethanolextractwas 84.42 ± 10.20 mgQE/g whiletheethylacetateextractwas (78.61 ± 5.31). Theseresults are in accordancewiththeresearchof[8]withthe total flavonoidscontentof*Caulerparacemosa*ofmethanolextract 114.16 ± 0.91 mgQE/g andchloroformextractof 86.33 ± 6.96 mgQE/g. Accordingtoresearchby[11]showedthattheflavonoidscontentof*Caulerpalentillifera*ofmethanol extractwas 116.82 mgQE/100g. Accordingto[28]showedthatthe total flavonoidsofethylacetateextractfrom*Gracilariasp*.seaweed had a valueof 25.23 ± 0.46 mgQE/g followedbyethanolextractwas 21.78 ± 0.32 mgQE/g. Whileaccordingto[40]showedthe total flavonoidsof*Eucheumacottonii*seaweedofethylacetateextractwas 35.17 ± 1.00 mgQE/g andmethanolextractof 17.78 ± 0.31 mgQE/g.

The resultsshowedthatthemethanolextractwashigherwhencomparedtotheethylacetateextract. In addition, *Caulerparacemosa*seaweed has a higher total flavonoidscontentwhencomparedto total phenol. Accordingto[28]flavonoidsthatbindtosugarstendtodissolve in water (polar), whileless polar aglyconessuch as isoflavones, flavones, flavonon, andflavonolstendtodissolvemoreeasily in semi-polar solvents. Flavonoids are oneofthe natural antioxidantsthathavethefunctionofinhibitingtheoxidationoflowdensity lipoprotein (LDL) whichis a triggerfornarrowingofbloodvessels. Accordingto[42]suggestedthat natural flavonoidscompoundsincludingkaempferol, miricetin, morin, andquercetinhaveprotectiveactivitybyreducing α-tocopherolcontent in LDL. Accordingto[17]flavonoids are a classofphenoliccompoundsthatformthebestantioxidantsfound in plants.

Antioxidant activity assay

Antioxidant activity is declared very strong if the IC50 value is < 50 µg/mL, strong 50-100 µg/mL, moderate 101-150 µg/mL, and weak 150-200 µg/mL [21]. Antioxidant activity testing of *Caulerpa racemosa* with methanol and ethyl acetate solvents using ABTS, DPPH, and H_2O_2 methods showed strong to moderate antioxidant activity, respectively. The control used in each test was ascorbic acid. The IC50 values in each test are as follows.

ABTS (*2,2-azinobis-3-Ethylbenzoathiazoline-6-sulfonic acid*) assay

The antioxidantactivityof*Caulerparacemosa* ABTS methodwithmethanolandethylacetatesolvents has freeradicalscavengingactivityshown in Figure 1 methanolextract has an IC50 valueof 134.64 ± 19.90 indicatingmoderateantioxidantactivity, whileethylacetateextract has a better IC50 valueof 113.50 ± 19.69 indicatingmoderateantioxidantactivity.

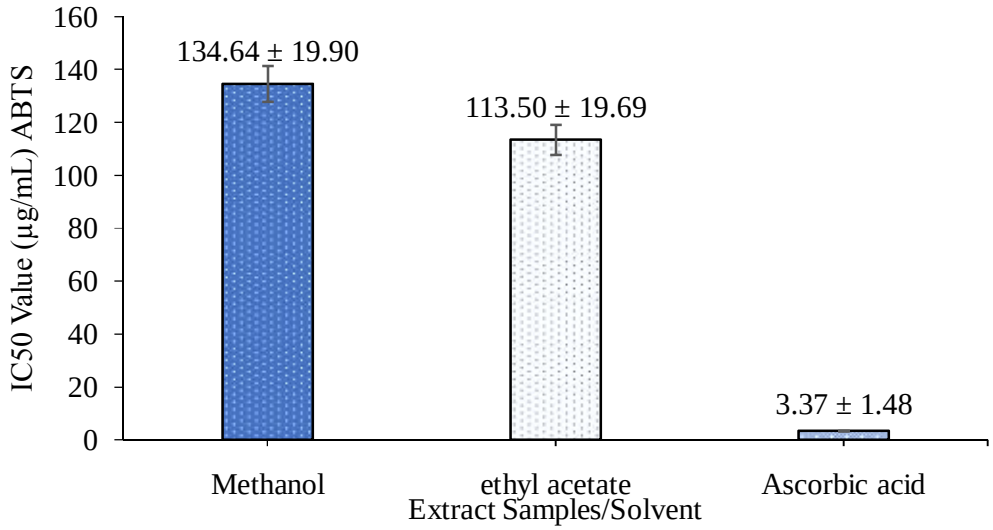

Figure 1.
Antioxidantactivityofmethanolandethylacetateextractsof*Caulerparacemosa*withcomparativea scorbicacidby ABTS method

Ethylacetateextractshowedbetterantioxidantactivitythanmethanolextract. Accordingto[17] in theirresearchshowedthatthemethanolextractof*Caulerpafiliformis* has goodantioxidantactivitywith EC50 valuesof 2.546 and 4.624. Mamani furtherexplainedthattheantioxidantcapacityof*Caulerpa* has beenstudied in theformofextractswithantioxidants, whichvary in thesameorsimilarspecies.

The*Padinasp*and*Sargassumsp*seaweedhavedifferentantioxidantactivities. The IC50 valueof*Sargassumsp*extractishigher (64.80-102.48 mg/L) whencomparedto*Padinasp*by (101.78-126.99) thedifference in IC50 resultsfromextracts in the ABTS testcanbeinfluencedbythetypeofsample, solvent, andextractionmethod[2].

DPPH (*2,2-diphenyl-1-pichrylhydrazyl*) assay

The antioxidantactivityof*Caulerparacemosa* DPPH methodwithmethanolandethylacetatesolvents has freeradicalscavengingactivityshown in Figure 2. The antioxidantactivityof DPPH methodofmethanolandethylacetateextractsof*Caulerparacemosa*iscategorized as strongtoweak. The methanolextract has a fairlygood IC50 valueandisclassified as a strongantioxidantat 96.08 ± 0.45 µg/mL, whiletheethylacetateextract has a muchlower IC50 valuewith a valueof 167.72 ± 1.08 µg/mLandisclassified as weakantioxidantactivity.

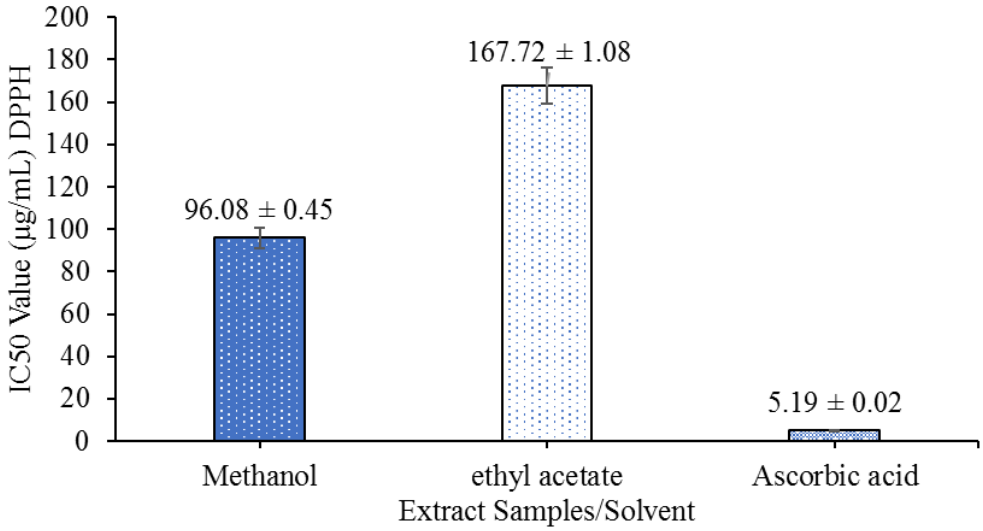

Figure 2. Antioxidantactivityofmethanolandethylacetateextractswithascorbicacidusing DPPH method

The difference in IC50 values in the DPPH methodantioxidanttestisduetodifferences in thesolventsused. Accordingto[11]explainedtheeffectivenessofantioxidants in methanolextracts in counteractingfreeradicalsisthoughttoberelatedtothe polar natureofmethanolsothatmanyphytochemicalcomponentsdissolve in it. Bioactivecompounds in *Caulerpalentillifera*thatact as antioxidants are alkaloids, flavonoidss, phenolics,

andsteroids/terpenoids. Accordingto[17]mentionedseveralstudieson*Caulerpa*alsoshowed a greaterabilityofextractsto trap freeradicals (DPPH).

Basedonthe IC50 valueofantioxidantactivity testing obtained, itshowsthatmethanolextract has thebest IC50 valuewith a strongcategorywhileethylacetateisweak. Theseresultshavebetterantioxidantactivitythantheresearchof[18]whichshowedtheantioxidantactivityof*Caulerpa*ofmethanolextract (IC50 = 132.08 µg/mL) included in themoderatecategory. Furthermore, [18]explainedthattheantioxidantactivitytest in inhibitingfreeradicalsisbasedontheabilityofaningredient/extracttodonateelectronsorhydrogencompoundsto DPPH freeradicalssothatmorestablefreeradicalswillbeformed.

H_2O_2 (*Hydrogenperoxide*) assay

Hydrogenperoxideis a hydroxylradicalthat has toxicpropertiesandcancausedamagetocellsandtissues in thebody[23].Thisassaymeasuresthereduction in hydrogenperoxide (H_2O_2) concentrationafterexposuretoplantextractsthathaveantioxidantpotential[16]. The hydrogenperoxidescavengingactivityofmethanolandethylacetateextractsof*Caulerparacemosa*isdescribed in Figure 3.

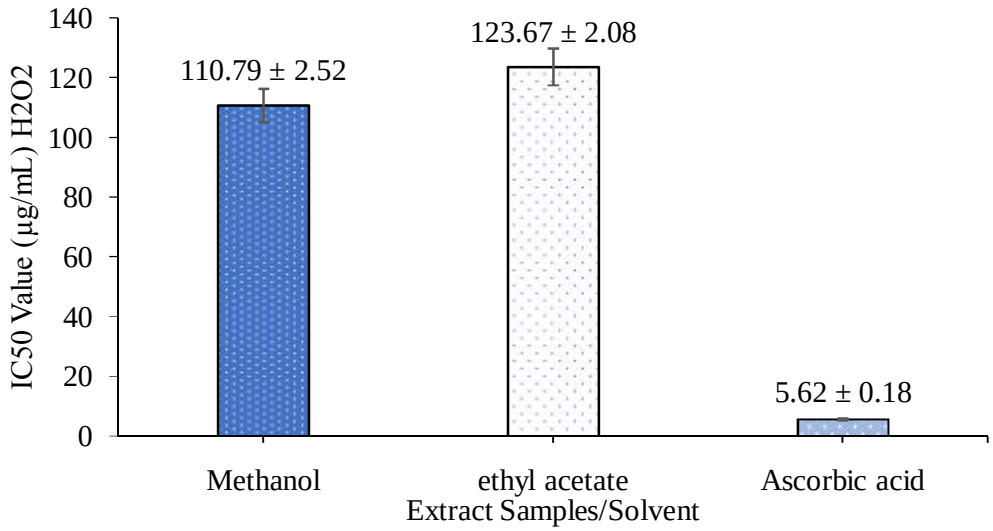

Figure 3.
Antioxidantactivityofmethanolandethylacetateextractsof*Caulerparacemosa*withcomparativeascorbicacid H_2O_2 method

H_2O_2 radicalscavengingactivity in methanolandethylacetateextractsshowed IC50 valueswithmoderatecategories, wheremethanolextracts (110.79 ± 2.52 µg/mL) had better IC50 valuesthanethylacetateextracts (123.67 ± 2.08 µg/mL). Methanolextracts had a percentageof H_2O_2 scavengingof 48.68% at a concentrationof 100 ppm, whileethylacetateextractsamountedto 47.96% at a concentrationof 47.96%. Theseresults are stilllowerwhencomparedtotheresearchof[23],wherethescavengingactivityof*Caulerpalentillifer*

*a*extractwiththermaldryingandfreezedryingrangesfrom 50-70%. While in [32]showedlowerscavengingactivity in methanolextract*Caulerparacemosa* (20.78%) and*Caulerparacemosa*varmacrophysa (24.91%) wheretheseresults are stillhigherwhencomparedtoothergreenseaweed, namely*Caulerpascalpelliformis*with a percentageofscavenging (17.64%), *Cladophoravagabunda* (16.71%) and*Ulva Lactuca* (16.32%).

CorrelationofTotal PhenolandTotal FlavonoidsContenttoAntioxidantActivity

Phenoliccompounds in macroalgaehavebeenwidelyreportedtohavepotential as natural antioxidants, butantioxidants in seaweed are not onlycausedbyphenoliccompounds[13]. The roleofphenoliccompounds as antioxidants has todowithconjugatebondsonthebenzenearomatic ring
andthenumberofhydroxylfunctionalgroups[35].Accordingto[17]theantioxidantactivityofmacroalgaecanbeattributedtothephenoliccontentwithoutrulingoutthesynergisticactionbetweenthesecompounds. Linear regressionanalysisbetween total phenoland total flavonoidscontenttotheantioxidantactivityof ABTS, DPPH, and H_2O_2methodsaimstodeterminetheclosenessoftherelationshipbetween total phenoland total flavonoidscontenttoantioxidantactivity.

The antioxidanttestof ABTS methodshowedthatethylacetateextractwasbetter in inhibiting ABTS freeradicalsthanmethanolextractinhibit ABTS freeradicalsthanmethanolextract. Basedon linear regressionanalysisthe total phenolcontentofmethanolextract (R^2 = 0.98) andethylacetate (R^2 = 0.96) has a verystrongrelationshiptotheantioxidantactivityof ABTS method. Furthermore, the total flavonoidscontentofextractmethanol (R^2 = 0.88) andethylacetate (R^2 = 0.97) also had a verystrongrelationshiptotheantioxidantactivityof ABTS method. soitisknownthatthecontentof total phenolsand total flavonoidsscanact as antioxidantactivity in counteracting ABTS radicals. Accordingto[33]the ABTS methodismoresensitive in detectingantioxidantconcentrations in lowlevels. lowlevels. ABTS testing canevaluateantioxidantcompoundsthatdo not performwell in the DPPH assay[26].

In testing theantioxidantactivityofthe DPPH method, themethanolextractwasbetter in inhibiting DPPH freeradicalsthantheethylacetateextract. Basedontheresultsof linear regressionanalysisshowedthe total phenolcontent in methanolextract (R^2 =0.97) andethylacetate (R^2 = 0.85) has a verystrongrelationshiptotheantioxidantactivityof DPPH method. At the total flavonoidscontentofmethanolextract (R^2 = 0.68) had a closerelationshiptotheantioxidantactivityoftheantioxidantactivityof DPPH method, onthecontrary, theflavonoidscontentofethylacetateextract (R^2 = 0.47) has a relativelyweakrelationship. Itissuspectedthatthe DPPH method has bettereffectiveness in polar solventssuch as methanolthanethylacetate in analyzingantioxidantactivitytoassessthecontentofphenoliccompoundsandflavonoidss[27,30].According to[26]the DPPH methodiseffective in assessinghydroxylgroupcompounds, whilesomephenoliccompoundssuch as dihydrochalconesandflavonesdo not reactwellwith DPPH. In accordancewiththeresultsofthis study wherethemethanolsolvent has a betteradvantage in attractingphenoliccompoundsthanethylacetate, whilethe total

contentofflavonoidss has moreresultsthan total phenols in bothsolvents. In accordancewith ABTS methodantioxidantactivity testing which has a betteradvantage in ethylacetatesolvent.

In testing theantioxidantactivityofthe H_2O_2method, methanolextractisbetter in removingorscavenginghydrogenperoxidethanethylacetatesolvent. Basedontheresultsof linear regressionanalysisshowedthe total phenolcontentofmethanolextract (R^2 = 0.84) andethylacetate (R^2 = 0.94) had a verycloserelationshiptoantioxidantactivityof H_2O_2 method. At the total flavonoidscontentofmethanolextract (R^2 = 0.99) andethylacetateextract (R^2 = 0.95) alsoshowed a verycloserelationshiptotheantioxidantactivityof H_2O_2 method. The correlationvalueshowsthattheflavonoidscompoundsofmethanolextracthave a verystrongrelationshiptotheantioxidantactivityofthe H_2O_2 method. Flavonoidscompounds are knowntoprotect in thebodycausedby H_2O_2. Accordingto[36]flavonoidss are significantlyabletoreducereactivatedoxygenlevels in theformof H_2O_2 in thebodyandincreasetheactivityofantioxidantenzymes. Flavonoidss in theformofquercetinandnaringinhavebeenshowntoprotect DNA fromoxidativedamagecausedby H_2O_2, showingtheirrole as effectivefreeradicalscavenger[38]. In otherstudiesexplainthespecificstructureofflavonoidsswithtwohydroxylgroups in ring B isessentialforinhibiting lipid peroxidation in human erythrocytesexposedto H_2O_2[9]. Accordingto[14] H_2O_2 is not soreactive, but in certaincases H_2O_2 canbetoxictocells in thebodybecauseitcancausehydroxylradicalsofcellnature.

Basedonthe ABTS and H_2O_2 testmethods, theincubationtimeisfasterthanthe DPPH methodsothatitcanshortenthetesttimebutthereagenttsneededmore, preparationofreagenttsandsamplesisquitecomplicatedandrequireslongertime in thepreparationofreagenttsthanthe DPPH method. Thisshowsthatthe DPPH method has theadvantageofsimplicityandcheapercost-effectiveness, butthe testing ofantioxidantactivityof ABTS methodismoresensitive in evaluatingcompoundswithlowlevels.

The choiceoftestmethodbetween ABTS, DPPH and H_2O_2 whichismoreeffectivedependsonthecharacteristicsoftheantioxidantcompoundstobetestedandth enatureofthesamplematrix. The compositionandstructureofantioxidantsaffectthereactionratesignificantly. Differences in reactionequilibriumtimecanaffectthewrongestimation. Itisimportanttodeterminetheappropriatetestmethodtoevaluatetheantioxidantcontentaccurately. Basedonthewavelengthused, antioxidantresultsobtainedvarybasedontheabsorptioncharacteristicsoftheantioxidantandthecon centration in themeasurementsystem[25].

4. CONCLUSION

The yieldof*Caulerparacemosa*extractsfromsolventswithdifferentpolaritiesgivesaninfluenceonthe total phenoland total flavonoidscontentobtained as well as theantioxidantactivity in variousmethods. Methanolsolventshowed a betterextractyield (6.12%) in 25 g sample. The total phenolcontentofthemethanolextractwas 40.18 ± 3.06 mgGAE/g, whilethe total flavonoidscontentofthemethanolextractwas 84.42 ± 10.20 mgQE/g. IC50 in the ABTS methodantioxidantactivityofethylacetateextract 113.50 ± 19.69 µg/mL. IC50

onantioxidantactivityof DPPH method in methanolextract 96.08 ± 0.45 µg/mL. IC50 ontheH$_2$O$_2$ scavengingactivityofmethanolextractwasobtained 110.79 ± 2.52 µg/mL.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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