

TABLE OF CONTENTS

	Pages
COVER PAGE	i
INNER COVER PAGE	ii
THESIS FULFILMENT	iii
APPROVAL PAGE	iv
EXAMINATION COMMITTEE	v
STATEMENT OF ORIGINALITY	vi
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF APPENDICES	xiii
LIST OF ABBREVIATIONS	xiv
SUMMARY	1
ABSTRACT	3
CHAPTER I INTRODUCTION	4
1.1 Background of the study	4
1.2 Problem of research	7
1.3 Objective of research	7
1.4 Benefit of research	8
CHAPTER II LITERATURE REVIEW	9
2.1 Description.....	9
2.1.1 Phytochemical of <i>Sargassum species</i>	10
2.1.2 Heavy metal of <i>Sargassum species</i>	10
2.2 Description	11
2.2.1 Phytochemical of <i>Padina species</i>	12
2.2.2 Heavy metal of <i>Padina species</i>	13
2.3 Spectroscopy	13
2.3.1 UV spectrophotometry	14
2.3.2 Atomic absorption spectrometry	14
2.4 Antioxidant compound in seaweed.....	15
Thesis The Antioxidant, Heavy Metal, Toxicity Content Nwet Darli Kyaw Zaw	

2.4.1 Phenolic compounds	15
2.5 Biological activity test	19
2.3.1 Antioxidant	19
2.3.2 Free radical and other mechanism	20
2.3.3 2,2-diphenyl-1-picrylhydrazyl radical Assay	22
2.3.4 <i>Folin-Ciocalteau (F-C) Assay</i>	23
2.4 Heavy metals.....	23
2.4.1 Effect of heavy metal in the environment.....	24
2.4.2 The heavy metal and antioxidant activity related in seaweeds	25
CHAPTER III THE RESEARCH CONCEPT	29
3.1 Conceptual framework	29
3.2 Hypothesis	31
CHAPTER IV RESEARCH METHODS	33
4.1 Time and place of research	33
4.2 Apparatus	33
4.3 Chemical reagent	33
4.4 Sample collection	33
4.5 Preparation of sample	34
4.6 Phytochemical screening of crude extracts.....	34
4.6.1 Test for terpenoids	35
4.6.2 Test for alkaloids	35
4.6.3 Test for flavonoids	35
4.6.4 Test for steroid	35
4.7 Determination of total phenolic contents	36
4.8 Determination of antioxidant activity by DPPH radical scavenging activity	36
4.8.1 Preparation of 0.1 M acetate buffer solution	36
4.8.2 Preparation of DPPH solution.....	37
4.8.3 Preparation of ascorbic acid solution	37
4.8.4 Preparation of test sample solution	37
4.8.5 Procedure	37

4.9 Determination of Heavy metal content	38
4.10 Determination of Toxicity content	39
CHAPTER V RESULT AND DISCUSSIONS	41
5.1 Phytochemical screening	41
5.2 Total phenolic content	41
5.3 DPPH radical scavenging assay	43
5.4 Heavy metal content	44
5.5 Toxicity content	45
CHAPTER VI CONCLUSION	48
7.1 Conclusion	48
7.2 Suggestion	49
REFERENCES	50

LIST OF TABLES

Table 2.1	Classification of tannin	18
Table 5.1	The phytochemical analysis of <i>S.duplicatum</i> and <i>P.tetrastromatica</i> from non-oil and oil extraction sites	41
Table 5.2	The total phenolic content (TPC) of <i>S. duplicatum</i> and <i>P.tetrastromatica</i> from non-oil and oil extraction sites	43
Table 5.3	The antioxidant activity of <i>S. duplicatum</i> and <i>P.tetrastromatica</i> from non-oil and oil extraction sites	44
Table 5.4	The heavy metal content of <i>S. duplicatum</i> and <i>P.tetrastromatica</i> from non-oil and oil extraction sites	45
Table 5.5	The toxicity content of <i>S. duplicatum</i> and <i>P.tetrastromatica</i> from non-oil and oil extraction sites	47

LIST OF FIGURES

Figure 2.1 The brown seaweed of *Sargassum duplicatum*.....9

Figure 2.2 The brown seaweed of *Padinatetrastromatica*.....12

Figure 2.3 Classification of flavonoid and their chemical structure16

Figure 2.4 Classification of phenolic acid17

Figure 2.5 Electron structures of common reactive oxygen species21

Figure 2.6 DPPH free radical conversions to DPPH by
antioxidant compounds23

Figure 2.7 Mechanisms involved in the tolerance to heavy metal
stress in seaweeds. ABC transporter (ABC);
calmodulin (CAM); chloroplast (*chl*); glutathione (GSH);
glutathione S-transferase (GST); heavy metals like Cd, Cu,
Pb, and others (M); polyphosphate bodies (PPBs);
reactive oxygen species (ROS); vacuole (*va*). Some enzymes
involved in these mechanisms are catalase (CAT),
ascorbateperoxidase (AP), peroxiredoxin (PRX),
andlipoxygenase (LOX), among others27

Figure 3.1 Conceptual framework of research32

Figure 4.1 (a) The location of Camplong beach, KabupatenSampang
(with oil extraction site) and (b) Jumiang beach, Pamekasan
(without oil extraction site) at Madura Island34

Figure 4.2 Flow chart extraction and determination
(heavy metal and antioxidant activity) of *S.duplicatum*
and*P.tetrastromatica* from different sites40

LIST OF APPENDICES

Appendices 1 The result of phytochemical content of *S.duplicatum* and *Padinatetrastromatica* in non- oil and oil extraction sites60

Appendices 2 Results of total phenolic content were analyzed by SPSS61

Appendices 2.1 The result of total phenolic content of *S.duplicatum* and *P.tetrastromatica* in non-oil and oil extraction site calculatedby excel standard deviation61

Appendices 3 Experimental of total phenolic content63

Appendices 4 Results of antioxidant activity were analyzed by SPSS63

Appendices 4.1 The result of antioxidant activity of *S.duplicatum* and *P.tetrastromatica* in non-oil and the oil extraction sites calculated by excel standard deviation64

Appendices 5 Results of heavy metal content were analyzed by SPSS66

Appendices 5.1 The data of heavy metal content of *S.duplicatum* and *P.tetrastromatica* in non-oil and the oil extraction sites calculated by excel standard deviation67

Appendices 6 Experimental of heavy metal69

Appendices 7 Results of heavy metal content were analyzed by SPSS71

Appendices 7.1 The data of toxicity content of *S.duplicatum* and *P.tetrastromatica* in non-oil and the oil extraction sites calculated by excel standard deviation73

Appendices 8 Experimental of toxicity content75

LIST OF ABBREVIATIONS

Hg	:Mercury
Pb	:Lead
Cd	:Cadmium
Cr	:Chromium
Ni	:Nickel
As	:Arsenic
Cu	:Copper
Zn	:Zinc
K	:Potassium
Co	:Cobalt
Ca	:Calcium
mg	:Milligram
kg	:Kilogram
L	:Liter
mL	:Milliliter
µg	:Microgram
µL	:Microliter
ROS	:Reactive oxygen species
RNS	:Reactive nitrogen species
RCS	:Reactive chlorine species
H ₂ O ₂	:Hydrogen peroxide
DPPH	:2,2-diphenyl-1-picrylhydrazyl
DNA	:Deoxyribonucleic acid
A ₀	:Absorbance of control
A ₁	:Absorbance in presence of sample extracts
nm	:Nanometer
g	:Gram
M	:Molarity
NaHCO ₃	:Sodium hydrogen carbonate
IC ₅₀	:50% Inhibition concentration
pH	:Potential hydrogen

ppt	:Parts per thousand
UV	:Ultraviolet
TPH	:Total phenolic content
%	:Percentage

Summary

Seaweeds are the primary producers of all aquatic ecosystems. They are large and diverse group of organisms which play vital ecological roles in marine communities. It falls into three broad categories as brown, red and green seaweed based on pigmentation. The Phaeophyceae or brown seaweeds are a large group of multicellular algae. Worldwide there are about 1,500 species of brown seaweeds and they produce vast numbers of useful bioactive components. In Indonesia there are many types of seaweed, including economic value is enough high like *Sargassum* and *Padina* brown seaweed. *Sargassum* and *Padina* species are very abundant and scattered wide in Indonesian waters. The purpose of this study is to determine the levels of heavy metal, phytochemical, antioxidant activity and toxicity in seaweed (*Sargassum duplicatum* and *Padina tetrastrumata*) through a DPPH test, phytochemical screening, AAS, and brine shrimp lethality test. This study compared between seaweeds from two different sites; Camplong beach at Sampang district and Jamiang beach at Tanjung Village, both are located in East Java Province of Indonesia by utilizing methanol solvent.

The antioxidant activity and total phenolic content from non-oil extraction site were generally greater than oil extraction site in both these species. The value of antioxidant activity showed by methanol extracts of *P.tetrastrumata* ($IC_{50} 53.5693 \pm 1.214 \mu\text{g/ml}$) and methanol extracts of *S. duplicatum* ($IC_{50} 265.91 \pm 1.358 \mu\text{g/ml}$) in non-oil extraction site. In case of the oil extraction site, the antioxidant activity of methanol extract from *P.tetrastrumata* ($IC_{50} 120.866 \pm 3.138 \mu\text{g/ml}$) and methanol extracts of *S.duplicatum* ($IC_{50} 1208.574 \pm 12.136 \mu\text{g/ml}$). Alkaloid, flavonoid and steroid were observed from both these species in two different sites. But, terpenoid was not found. In both these species, the total phenolic content was observed from non-oil extraction site (24.67 ± 3.47 and $102.36 \pm 5.77 \text{ mg/g}$) was greater than compared with those that obtained from oil extraction site (15.95 ± 0.44 and $89.28 \pm 7.74 \text{ mg/g}$).

In the case of Cd, both these species were higher in oil extraction site than in non-oil extraction site. The maximum level of Cd $0.382 \pm 0.09 \text{ mg/kg}$ from *P.tetrastrumata* was recorded in oil extraction site and a minimum of $0.157 \pm 0.05 \text{ mg/kg}$ in non-oil extraction site. Similarly, *S. duplicatum* was observed $0.2511 \pm 0.170 \text{ mg/kg}$ in oil extraction site and a minimum of $0.1337 \pm 0.015 \text{ mg/kg}$ in non-oil extraction site. The amount of Cu in *P.tetrastrumata* was also higher in oil extraction site than in non-oil extraction site. The amount of Cu in *P.tetrastrumata* was found to be a maximum of $0.741 \pm 0.211 \text{ mg/kg}$ in oil extraction site and a minimum $0.056 \pm 0.008 \text{ mg/kg}$ in non-oil extraction site. Similarly another species, *S. duplicatum* was not found in non-oil extraction site and oil extraction site. Similarly, *S. duplicatum* was not found in non-oil extraction site and oil extraction site. Briefly, Pb concentration were not observed from both *P.tetrastrumata* and *S. duplicatum* seaweed in non-the oil extraction site and oil extraction site.

The higher toxicity content for both species were also observed in oil extraction than non-oil extraction. The highest toxic content in *P.tetrastrumata* from oil extraction site in $1000 \mu\text{g/ml}$ concentration was observed $30 \pm 10 \%$ at 24 h and $57 \pm 6 \%$ at 48 h than which that are from the non-oil extraction site found $13 \pm 6 \%$ at 24 h and $37 \pm 6 \%$ at 48 h. Similarly, *S.duplicatum* was found $37 \pm 6 \%$ at 24 h and 70

$\pm 10\%$ at 48 h from oil extraction site in $1000\ \mu\text{g/ml}$ concentration than which are from non- extraction site in $1000\ \mu\text{g/ml}$ was observed $23\pm 6\%$ at 24 h and $40\pm 40\%$ at 48 h from the non-oil extraction site. According to this research, the two species is useful in pharmacological preparations. The research can give suggestion to do isolation of bioactive compounds, determination bioactivities in vivo and vitro, creating cosmetic product and making food because of less toxic heavy metal and toxicity in future.