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LIST OF ABBREVIATIONS

µg	:	microgramme
µg/mL	:	microgramme per millilitre
µL	:	microlitre
A. hydrophila	:	*Aeromonas hydrophila*
AAPH	:	2,2'-azobis-(amidinopropane) dihydrochloride
Abs	:	absorbance
ACH_{50}	:	alternative complement pathway haemolytic activity
AIDS	:	acquired immune deficiency syndrome
BHT	:	butylated hydroxytoluene
C	:	Carbon
C_6H_{14}	:	hexane
Ca	:	Calcium
CAT	:	catalase
CCl_4	:	Carbon tetrachloride
CFU	:	colony forming units
$CHCl_3$	:	Chloroform
Cl	:	Chlorine
CL	:	chemilumnescence
Cl	:	Chlorine
Co	:	Cobalt
-COOH	:	carboxylic acid
Cox	:	cyclooxygenase
Cu	:	Copper
Cu	:	Copper
DNA	:	deoxyribonucleic acid
DO	:	dissolved oxygen
DPPH	:	1, 1-diphenyl-2picrylhydrazyl

EtOAc	:	ethyl acetate
FAO	:	Food and Agriculture Organization
Fe	:	Iron
Fe^{2+}	:	Ferrous ion
$FeCl_3$	:	Iron III chloride
g	:	gramme
g/L	:	gramme per litre
GLA	:	gamma lenolenic acid
GPx	:	glutathione peroxidase
HCl	:	hydrochloric acid
HIV	:	human immunodeficiency virus
-HOCl	:	hypochlorous acid
HPLC	:	high performance liquid chromatography
IC_{50}	:	inhibitory concentration
IgM	:	immunoglobulin M
K	:	Potassium
L	:	litre
LPS	:	lipopolysaccharides
m	:	Metre
M	:	molar
M. oleifera	:	*Moringa oleifera*
MC	:	chloroform fraction *M. oleifera*
Mcrude	:	crude extract of *M. oleifera*
MDA	:	melondialdehyde
MEA	:	ethyl acetate fraction of *M. oleifera*
MeOH	:	methanol
Mg	:	Magnesium
mg	:	Milligramme

mg/kg	:	milligramme per killogramme
mg/L	:	milligramme per litre
mg/mL	:	milligramme per millilitre
MH	:	hexane fraction of *M. oleifera*
min	:	Minute
mL	:	Millilitre
MM	:	methanol fraction of *M. oleifera*
mm	:	Millimetre
mM	:	milliMolar
MSPC	:	chloroform fraction of *S. platensis* + chloroform fraction of *M. oleifera* in (1:1)
MSPcrude	:	crude extract of *S. platensis* + crude extract of *M. oleifera* (1:1)
MSPH	:	hexane fraction of *S. platensis* + hexane fraction of *M. oleifera* (1:1)
MSPM	:	methanol fraction of *S. platensis* + methanol fraction of *M. oleifera* in (1:1)
N	:	Nitrogen
Na	:	Sodium
NaCl	:	Sodium chloride
NCDs	:	non-communicable diseases
NDDS	:	novel drug delivery system
NH^{+4}	:	ammonium
Ni	:	Nickel
NO^{2-}	:	nitrate
NO^{3-}	:	nitrate
NPK	:	Nitrogen Phosphorus Potassium fertilizer
$-OCH_3$	:	methoxyl group

-OH	:	hydroxyl group
$-ONOO^-$	:	peroxynitrite anion
P	:	Phosphorus
PGs	:	prostaglandins
pH	:	Potential hydrogen
$PO3^{-4}$	:	phosphate
ppm	:	part per million
ppt	:	part per thousand
RNA	:	ribonucleic acid
ROS	:	reactive oxygen species
S	:	Sulphur
S. aureus	:	*Staphylococcus aureus*
S. platensis	:	*Spirulina platensis*
Se	:	Selenium
SOD	:	superoxide dismutase
SPC	:	chloroform fraction of *S. platensis*
SPcrude	:	crude extract of *S. platensis*
SPEA	:	ethyl acetate fraction of *S. platensis*
SPH	:	hexane fraction of *S. platensis*
SPM	:	methanol fraction of *S. platensis*
TEM	:	Transmission Emission Microscopy
TFC	:	total flavonoid content
TPC	:	total phenolic content
UN	:	United Nations
V. alginolyticus	:	*Vibrio alginolyticus*
Zn	:	Zinc
γ-LFA	:	gamma linolenic fatty acid

SUMMARY

Aondohemba Samuel Nege. 2020. Antioxidative and Antibacterial Activity of *Spirulina platensis*, *Moringa oleifera* and their Synergies. Master of Fisheries and Marine Biotehnology, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya.

It is substantially true that both *S. platensis* and *M. oleifera* are high quality natural products in terms of human nutrition and health. *S. platensis* is one of the richest aquatic natural products while the latter is one of the richest on land but incidentally, both are natural commodities dominant in the tropics/sub-tropics and have both been reported by different scientists as good sources of antioxidants and antibacterial.

However, no previous study has compared the *in vitro* antioxidant and antibacterial activity of these natural products and their synergies. Hence, there are some questions about the antioxidant and antibacterial potentials of these two products that have not been answered in an individual research and these include: "which among the two products is higher in antioxidant activity"? "Which of the two is higher in antibacterial activity"? "Does synergism exist in terms of antioxidant and antibacterial activity when the extracts and fractions of *S. platensis* and *M. oleifera* are mixed to form a complex"?

Therefore this thesis was aimed at *Spirulina platensis* cultivation and comparison with *Moringa oleifera* as well as to test their synergistic effect in terms of antioxidant activity, phytochemical identity and antibacterial activity. The cultivation was performed outdoor using self-made artificial seawater. Water quality parameters revealed pH ranging from 8.00-10.00, dissolved oxygen 3.10-3.92mg/L, temperature 25.23-29.00^{o}C, light -46.33-3418.67Lux and salinity 15.67-29.33ppt during the 4week period. Pond water ammonium, nitrate, nitrite and phosphate ranged from 1-5mg/L, 0-25mg/L, 0.15-1.00 and ≥3.00mg/L respectively. The dried products were macerated and then fractionized. In the DPPH (1, 1-diphenyl-2picrylhydrazyl) antioxidant test, significantly higher DPPH inhibitions were observed in the crude mixture (MSPcrude) and hexane-fractional mixture (MSPH) of *S. platensis* and *M. oleifera* compared to their singles fractions.

However, the un-combined methanol and chloroform fractions of *M. oleifera* were significantly higher than those of their mixtures (MSPM and MSPC respectively). The ethyl acetate fraction from *M. oleifera* came 3rd in DPPH inhibition but the solvent failed to fractionise *S. platensis* sample. The lowest inhibitory concentration (IC_{50}) recorded was 182.57µg/mL, obtained in MSPcrude. Qualitative phytochemical identification revealed moderately high alkaloids (++) in SPcrude, not detected in Mcrude but present (+) in MSPcrude. Flavonoids and phenols were present (+) in Spcrude, moderately high (++) in Mcrude and MSPcrude while terpenoids were present (+) in both SPcrude and Mcrude but moderately high (++) in MSPcrude.

Same crudes and fraction with respective mixtures were tested against *Aeromonas hydrophila*, *Vibrio alginolyticus*, *Bacillus cereus* and *Staphylococcus aureus* at concentrations of 10, 20, 30, 40 and 50mg/mL respectively using the agar well diffusion method. On *A. hydrophila*, the inhibition zone of ethyl acetate fraction of *M. oleifera* (22.10±0.87mm) was highest, Moringa chloroform fraction (MC) had 19.94±2.90mm and the mixture (MSPC) with 19.22±1.16mm, Moringa methanol fraction (MM) with 16.46±1.93mm and combined methanol fractions (MSPM) with 14.06±2.36mm did not differ significantly with their respective mixtures, but both significantly differed from the Spirulina fractions alone (SPC and SPM). The hexane mixture (MSPH) with

11.56±1.33mm significantly differed from Moringa and Spiruina hexane fractions (MH and SPH) at 50mg/mL while the rest treatments indicated resistance (≤10mm).

The inhibition zone on *V. alginolyticus* was 10.26±1.85mm for Spirulina methanol fraction (SPM) and was significantly higher than MM and MSPM while the remaining 12 treatments were less than 10 mm at 50 mg/mL. Spirulina and Moringa crude extracts (SPcrude and Mcrude) were significantly higher than their combination (MSPcrude) on both *B. cereus* and *V. alginilyticus* bacteria. The ethyl acetate fraction of Moringa (MEA) had 13.80±6.02mm, Spirulina-Moringa chloroform fraction (MSPC) 11.58±4.10, Moringa chloroform fraction (MC) 11.02±12.86, Spirulina crude extract 10.32±2.82mm with the rest less than 10mm on *B. cereus* at 50 mg/mL.

Spirulina hexane fraction (SPH) had 11.08±1.21mm and was higher than MSPH and MH on *S. aureus* but not significant. The Moringa chloroform fraction had 10.52±1.85mm inhibition zone but was not significantly higher than SPC or MSPC. The crude mixture (MSPcrude) was statistically higher than Mcrude but not Spcrude, the crude extract inhibition zone and the rest treatments were less than 10mm against *S. aureus*. In conclusion, *S. platensis* and *M. oleifera* demonstrated antioxidant and antibacterial activity, and synergism in this study. The synergistic effects observed in this study is a new finding *in vitro* from *S. platensis* and *M. oleifera* which can be useful in the development of antioxidants, as well as combinational therapies and safer antibiotics in aquaculture, however further studies can be performed for additional confirmations.