International Journal of Agricultural Technology 2016 Vol. 12(7.2):2113-2122
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Diversity and Screening of Biological Activity of Red Macroalgae from Trang Watershed Area, Thailand

Wanninee Chankaew[*], Walapa Luaelae, Sampan Phromhom

Department of Fisheries, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, 80110, Thailand

C. Wanninee, L. Walapa and P,Sampan (2016). Diversity and screening of biological activity of red macroalgae from Trang watershed area, Thailand. International Journal of Agricultural Technology 12(7.2):2113-2122.

Freshwater red macroalgae were found in several places in Thailand. This research was aimed to measure in diversity and biological activities of some freshwater red algae from Trang watershed area. Diversity of red macroalgae were surveyed at 15 sites from Trang watershed area, southern part of Thailand. Twelve species of red algae in 11 genera *viz*., *Audouniella*, *Balliopsis*, *Batrachospermum*, *Bostrychia*, *Caloglossa*, *Compsopogon*, *Kumanoa*, *Nemalionopsis*, *Sirodotia*, *Sterrocladia* and *Thorea* were found. *Caloglossa ogasawaraensis* and *Compsopogon aeruginosus* extracts were obtained with ethanol and water, and screened for antioxidation activities using DPPH free radical scavenging. The total phenolic content of the extract was determined according to the Folin-Cioealteu method. The ethanolic extract of *Cologlossa ogasawaraensis* showed better radical-acavenging, while the highest of phelonic content was found in ethanolic extract of *Compsopogon aeruginosus*. There were not correlated between the DPHH scavenging activity and total phenolic content of all dry extracts. This results indicated that these algae could be potential candidates for development of antioxidants.

Keywords: red algae, Thailand, antioxidation

Introduction

There are more than 200 species of red algae only present in freshwater (Kumano, 2002). Freshwater red macroalgae were considered to be any algae visible to the naked eyes and recognizable in the field. The study on freshwater red algae in South-East Asia is not as well documented in terms of diversity and distribution data. Most of these studies focus on green and blue green macroalgae. Therefore, more studies on diversity of red mcroalgae are still urgently needed, especially in new community ecology. Such information could provide a baseline for stream management in the future, as well as applied aspects of the uses of red macroalgae.

[*] **Coressponding Author:** C. Wanninee, **E-mail:** wannaneeja@yahoo.com

Free radicals were found to be a product of normal metabolism in the body. The free radicals can damage and cause molecular and gene mutations leading to several disease conditions (Storz, and James, 1999). Macroalgae possess a variety of biological activities, including anti-inflammatory, anticancer, anti-HIV, antimutagenic and scavenging of free radicals (Shalaby, 2015). The bioactive compounds in various algae such as carotenoids, phenolics and sulphated polysaccharides have been shown to have antioxidant activities. Many researchers have become more interested in natural source which could block or reduce free radicals. Tipnee *et al*.,(2015) and Thumvijit *et al*.,(2013) have reported that extract of green macroalga, *Spirogyra varians* and *S. necleta*, respectively, have potential as a natural antioxidant.

In the Trang watershed area, southern part of Thailand, especially, filamentous algae, *Caloglossa* and *Compsopogon* are dominant benthic plants. These algae are predominantly found attached to rocky and stony shores or mixed with other genera such as *Spirogyra* and *Cladophora*. In this study, we determined the diversity of red algae and screen the total phenolic, the 2,2 – diphenyl-1picrylhydrasyl(DPPH) radical scavenging activity of *Caloglossa ogasawaraensis* and *Comsopogon aeruginosus* extract in order to understand the usefulness of both algae as a foodstuff as well as cosmeceutical products.

Materials and methods

***Study sites*:** Trang watershed area, located in southern part of Thailand (fig.1). The total area is 3,4335.57 km^2. The Trang watershed area contains one of the most important rivers in Trang province which originates from the Khao Luang Range in Nakhon Si Thammarat province and flows through Thung Song municipality before passing through Kantang district,Trang province. In this research, we selected 15 stream segments sites, list of sites as shown in Table 1. The red algae samples were scraped from the stones in the bottom of stream. The bottom of stream contained sand, gravel and stone. The sampling site was located in a forest with high percentage of canopy cover of the stream.

Red macroalgae sampling and Identification: The samples were collected from substrate *viz.* rocks and cobble, during dry season during February 2015 to March 2016). Specimen were kept in plastic boxes at low temperature. The algae were preserved in 2 % glutaraldehyd and subsequently taken back to the laboratory for species classification using morphology. Samples were identified following the relevant books and publications *viz*. Kumano (2002), Traichaiyaporn *et al*., (2003;2004;2008;2012) and Necchi and Vis (2012).

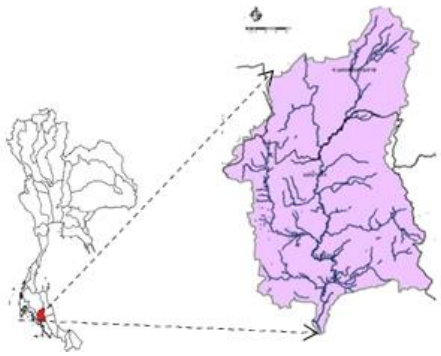
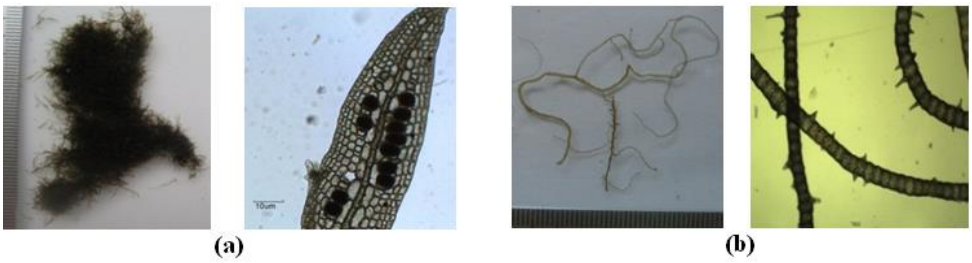


Fig 1. Map of Thailand showing the location of Trang watershed area.(Adapted from Vorrotnchaiphain, 2014)

Preparation of extracts **:** Two species of red macroalgae, *Caloglossa ogasawarensis* and *Compospogon aeroginosu*s (fig.2) were washed thoroughly with running tap-water to remove salts, sand and epiphytes. They were dried at 50 ℃ and then milled. The milled algae were subsequently used for preparing the extracts. As modified from those of Chew *et al*., (2008), the milled algae of each species was extracted with ethanol and water by continuously shaking for 24 h. The extracts were filtered through filter paper Whatman No. 1. The solvent was evaporated and the filtrate was concentrated by rotary evaporator. Next, the extracts were lyophilized to obtain dried powder for water extract.



(a) (b)

Caloglossa ogasawarensis Okamura *Compospogong aeroginosu*s (J. Agard) KÜtzing

Fig 2. Dominant species of red macroalgae, *Caloglossa ogasawarensis* (a), *Compospogon aeroginosu*s (b) in Trang watershed area, Thailand.

***Measurement of total phenolic contents*:** The total phenolic content (TPC) of each extract was measured using Folin-Ciocalteu method as described by Lopez *et al*., (2011). Briefly, 0.5 ml of diluted Folin-Ciocalteu reagent (1:9 v/v; Folin- Ciocalteu reagent: distilled water) was mixed with 100 µl of sample and was left at room temperature for 60 minutes. Then 1.0 ml of 20% sodium carbonate solution was added. After mixing, it was allowed to stand at room temperature for 60 minutes. The total phenolic content was determined using a microplate reader at 765 nm. Gallic acid was used as the standard, and the total phenolic content was expressed in terms of mg Gallic acid equivalents (GAE) per 1 g of extract.

***Measurement of antioxidant activities*:** The DPPH (1,1-diphenyl-1-picrylhydrazyl) radical scavenging activity of the algae extract was measured by the method described by Shimada et al., (1992). Briefly, 2.0 ml of 0.1 mM DPPH in ethanol was added to samples of 200 µl of the different algae extracts. The mixtures was shaken vigorously then allowed to stand at room temperature for 30 minutes before the absorbance was read at 517 nm. Butylated hydroxytoluene (BHT) and Ascorbic acid were used as a positive control. The capability to scavenge the DPPH radical was calculated using the following equation: Scavenging (%) = $[(A_{control} - A_{sample}/ A_{control}) \times 100]$, where $A_{control}$ is the absorbance of DPPH alone, and A_{sample} is the absorbance of the reaction mixture containing DPPH and sample.

***Statistical analysis* :** Data were expressed as the means $\pm$ SD of three measurements and analyzed using one-way ANOVA by Duncan test. Differences in mean values were considered significant when $P<0.05$.

Results and Discussion

Diversity of red macroalgae

On the biodiversity red algae, 12 species belonging to 11 genera that are distributed among the families Acrochaetiaceae, Batrachospermaceae, Compsopogonaceae, Thoreaceae,
Ceremiaceae, Caulacanthaceae and Delesseriaceae were recorded in all collected. Of the 10 genera *i.e*. *Audouniella*, *Compsopogon*,*Compsoponopsis*, *Batrachospermum*, *Kumanoa*, *Sirodotia*, *Thorea*, *Balliopsis*, *Sterrocladia* and *Caloglossa* were found. List of red algae and distribution in each study site are shown in Table 1. Most of them were found one species per site, while T8 site, where 4 taxa were found, had the highest richness values (Table 1). *Caloglossa ogagasawaraensis* as the widest distribution. During this study, maximum

algae density was recorded in March-April 2016 at T5 and T8 site, which the dominant species were *Caloglossa ogasawaraensis* and *Compospogon aeruginosus* respectively.The highest in amount of macroalgae observed in the dry season could be due to the nutrient run off during the rainy season, especially, in North East Monsoon, might influence growth and abundance of red macroalgae. There are 12 species in this study which is more than the number of algae species reported in Mae Hong Son province which consisted of 5 species in 3 genera (Traichaiyaporn *et al*., 2007). Interestingly, among these numbers, 1 red macroalgae species, *Bostrychia flagellifera* Post, which was new record for Thailand. Up to now, 52 freshwater red algae taxa were reported which belonged to 9 families and 15 genera in Thailand. According to 51 freshwater red algae taxa were previously reported.

Table 1 List of red mcroalgae, site distribution and frequency occurrence (%) in stream of Trang watershed area,Thailand.

Order	Family	Genus/species	site distribution	%frequency of occurrence (%)
Acrochaetiales	Acrochaetaceae	*Audouninella subtilis*	2	*
Batrachospermales	Batrachospermaceae	*Batrachospermum hypogynum*	12	*
		Kumanoa gibberosa	8	*
		Sirodotia suceica	8,12,13	**
Balliales	Ceramiaceae	*Balliopsis prieurii*	11,13	**
Compsopoganales	Compsopogonaceae	*Compsopogon aeruginosus*	1,8,9	**
Ceramiales	Delesseriaceae	*Caloglossa ogagasawaraensis*	1,2,3,4,5,68,9,13,15	*******
	Rhodomelaceae	*Bostrychia flagellifera*	6	*
Gigartinales	Caulacanthaceae	*Sterrocladia* sp.	6	*
Thoreales	Thoreaceae	*Thorea clavata*	2,10	**
		Thorea siamensis	2	*
		Nemalionopsis shawii	7	*

(* = <10%, ** = 11-20%, *** = 21-30%, **** = 31-40 %, ********** = >90%)
*abbreviations of sampling, 1=Klongnai, 2=Thrantip, 3=Taravarin, 4=Klongnumkaw, 5=Naitoa,6=Nansawan, 7=Rantong, 8=Plew, 9=Pakprag, 10=Wangping, 11=Nanplew, 12=Yong, 13=Klongthapaetai, 14=Naiwang, 15=Khaoklai

Extraction yield

Significant variations in extraction yield were found among different red algae (fig.3). The highest extraction yield was recorded for the water extract of *Caloglossa ogasawaraensis* which had higher yield of 14.77 % , followed by the ethanolic and aqueous of *Compsopogon aeruginosus* (4.55% and 3.55%, respectively).

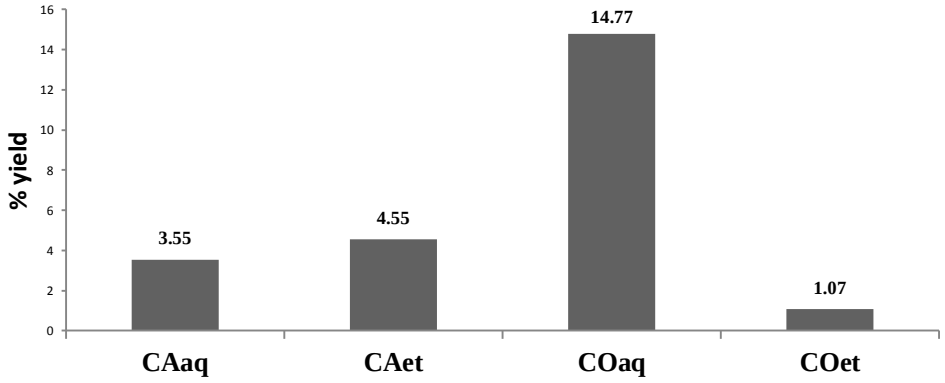


Fig 3. Yield (% w/w of dry weight) of red macroalgae extracts with different solvents.: CO = *Caloglossa ogasawaraensis*, CA= *Compsopogon aeruginosus*, aq = water extract, et = ethanol extract

Determination of total phenolic content

Phenolic compound are the class of antioxidant agents which act as free radical terminators. Total phenolic compounds were reported as gallic acid equivalents by reference to standard curve ($y = 0.9782x$, $r^2=0.9934$). The linear range range was within 0.05-1.0 mg L^{-1}. Eaeh point of the calibration curve is the average if three absorbance mearurements. In this study, total phenolic content analysis of the extracts revealed distinct different chemical profiles between the two algae. The total phenolic varied from 2.748 ± 0.12 to 28.502 ± 1.05 mg GAE g^{-1} dry extract. Ethanolic of *Compsopogon aeruginosus* with total phenol contents of 28.502 ± 1.05 mg GAE g^{-1} dry extract had the highest amount among the algae in this study (Table 2).

There are different amount of phenolic content in the freshwater macroalgae in Thailand, for example, in this results revealed both red macroalgae contained higher amounts of polyphenols (aqueous) than aqueous extract of *Nostoc commune* and *Cladophora* spp. ,which the total phenolic

were 0.64 ± 0.002 and 0.578 mg GAE g^{-1} dry extract, respectively (Yucharoen *et al*., 2015; Amornlerdpison *et al* ., 2015).

Table 2. Total phenolic content in the algae extracts

algae species	Total phenolic content (mg GAE g^{-1} extract)
aqueous of *Compsopogon aeruginosus*	6.143 ± 0.43^{a}
ethanolic of *Compsopogon aeruginosus*	28.502 ± 1.05^{d}
aqueous of *Caloglossa ogasawaraensis*	20.868 ± 0.68^{c}
ethanolic of *Caloglossa ogasawaraensis*	7.838 ± 0.11^{b}
ethanolic of *Caloglossa ogasawaraensis*	7.838 ± 0.11^{b}

Values are expressed as mean ± stand deviation, n=3, different superscript alphabets in each row, mean significantly different; $\alpha = 0.05$

DPPH radical scavenging activity

DPPH is the free radical that accept an electron or hydrogen radical to become a stable molecule and stable nitrogen-centered free radical. DPPH stable free radical method is an rapid and sensitive way to survey the antioxidation activity of a specific algae extracts (Shon *et al.*, 2003). The reduction capability of DPPH radical was determined by the decrease induced by antioxidative compounds and those results are shown in fig. 4.and 5. The lowest values of DPPH radical scavenging activity was observed in aqueous of *Compsopogon aeruginosus*. Similar to the phenolic content, the radical scavenger level varied with extraction method and type of red macroalgae. Among them, the ethanolic extract of *Caloglossa ogasawaraensis* showed the highest scavenging activity (IC_{50}=0.124±0.015mg mL^{-1}) and aqueous *Caloglossa ogasawaraensis* also exhibited similar scavenging activity (IC_{50}=0.183± 0.06 mg mL^{-1}) on DPPH free radical. This study, indicated that the antioxidant compounds were significantly different ($p<0.05$) depending on the species of freshwater algae. In addition, both aqueous and ethanolic of *Compsopogon aeruginosus* showed less in DPPH free radical scavenging activities than *Caloglossa ogasawaraensis*. The radical scavenging activity of *Caloglossa ogasawaraensis* could be due to the presence of folic acid, ascorbic acid and selenium. Moreover, of the tested samples, BHT and Ascorbic acid, positive control recorded a little of IC_{50}, lower IC_{50} value indicated higher antioxidant activity, which indicated that all % DPPH scavenging activities observe were significantly lower than of the BHT IC_{50}=0.038 mg mL^{-1}) and Ascorbic acid IC_{50}=0.0.041 mg mL^{-1}), positive control at the same concentration (fig. 4 and 5).

The high scavenging property of *Caloglossa ogasawaraensis* may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as radical scavenger. According to our study, the high contents of these phenolic in *Caloglossa ogasawaraensis* can explain its high radical scavenging activity (DPPH) in this algae. This study, indicated that the antioxidant compounds were significantly different ($p<0.05$) depending on the species of algae (Fig.4). In addition, the selection of the extracting solvent is an important factor for obtaining active compounds in algae. There fore further study can be done to select the best solvent. There are low correlation between DPPH values and phenolic contents in algae extract indicated that not only the phenolic compounds were involved in the antioxidant activity through this pathway but there could be some effects involving other active compounds (Lopez *et al*., 2011).

In conclusion, both the aqueous and ethanolic extracts of the examined red macroalgae contain antioxidative compounds that can strongly scavenge DPPH free radical. Therefore, it is suggested that further works should be performed on the isolation and identification of the antioxidant component in algae. The result from this study demonstrated the antioxidant activity of both, *Caloglossa ogasawaraensis* and *Compsopogon aeruginosus***.**

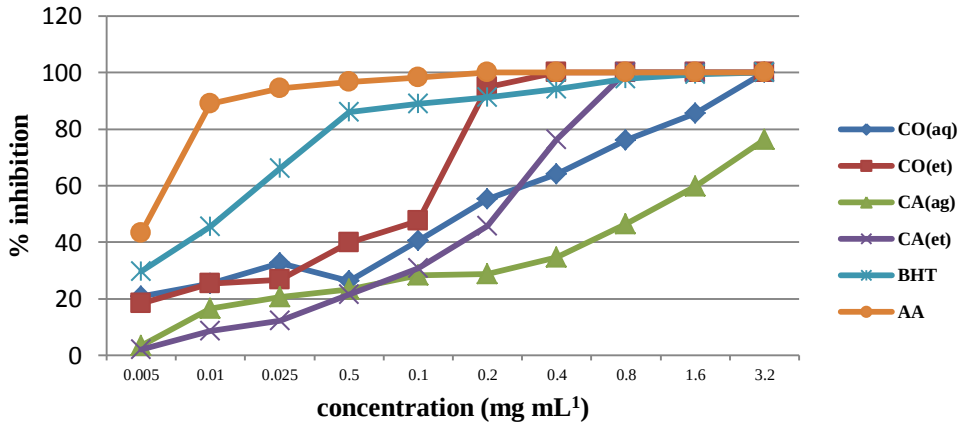


Fig 4. Values of algae extracts for free radical scavenging activity by DPPH radical.: CO = *Caloglossa ogasawaraensis*, CA= *Compsopogon aeruginosus*, BHT= Butylated hydroxyl toluene, AA= ascorbic acid

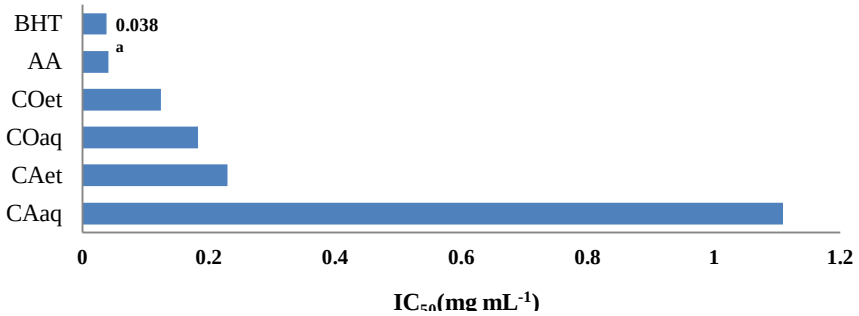


Fig 5. DPPH radical scavenging activity expressed in IC_{50} given in mg mL^{-1} (mean± SD; n=3) for algae extracts, CO= *Caloglossa ogasawaraensis*,CA= *Compsopogon aeruginosus*, BHT= Butylated hydroxyl toluene, AA= ascorbic acid

Conclusion

A total of 12 red macroalgae taxa were found in Trang watershed area, Thailand. This work represent screening of antioxidation activity of *Caloglossa ogasawaraensis* and *Compsopogon aeruginosus* extracts. These species which were from Trang watershed area, *Compsopogon aeruginosus* showed good but different levels of antioxidant activities in models studied. Phenolic contents were very good amount and higher than in some freshwater algae such as *Cladophora* spp. and *Nostoc commnne*. DPPH scavenging activity showed potent activity. Also, the extract of *Caloglossa ogasawaraensis* show more potent activity than *Compsopogon aeruginosus*. Identification of the antioxidant compound in this study will lend to their evaluation in considerable commercial potential in food production and in the cosmetic industry

Acknowledgement

This work was partially supported by grant from the Research Council of Thailand (2016). We would like to thank the students from Fisheries department, faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat campus for field assistance.

References

Amornlerdpison, D., Ngernjan, M., Mengumphan, K. and Junthin,R. (2016). Active compounds and oxidative defense of *Cladophora* spp. in hybrid catfish. KMUTT research and development journal 38(4): 393-405.

Chew, Y.L., Lim, Y.Y., Omar, M. and Khoo, K.S., (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci Technol. 41: 1067-1072.

Kumano, S. (2002). Freshwater red Algae of world. Biopress Limited. Bristol, England.
Laungsuwan, R. and Chulalaksananukul, W. (2013). Antioxidation and anticancer activities of freshwater green algae, *Microspora floccosa* and *Cladophora glomerata*, from Nan River in Northern Thailand. Maejo international Sciene Technology 7(02): 181-188.
Lopez, A., Rico, M., Rivero, A., and Tang, M.S., (2011). The effects solvents on the phenolic contents and antioxidant activity of *Stypocaulon scoparium* algae extracts. Food Chemistry 125: 1104-1109.
Necchi, J.O. and Vis, M.L. (2012). Monograph of the genus *Kumanoa* (Rhodophyta, Batrachospermales). Bibliotheca Phycologica, 116: 1-79**.**
Shalaby, E.A. (2015). Algae as a natural source of antioxidant active compounds. *pp.*129-147. *In* Plants as a source of natural antioxidants, Banaras Hindu University, India.
Shimada, K., Fujikawa, K.,Yahara K. and Nakamura T. (1992). Antioxidative proper of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. Journal of agicultural and food chemistry 40: 945-948.
Shon, M.Y., Kim, T.M and Sung, N.J., (2003). Antioxidants and free radical scavenging activity of *Phellinus baumii*(*Phellinus* of Hymenochaetoceae) extracts. Food chemistry 82: 593-597.
Storz, G. and James, A.I.(1999). Oxidative stress. Current opinion in Microbiology. 2: 188-194.
Thumvijit, T. Inboot, W., Peerapornpisal,Y., Amornlerdpisan, D. and Wongpoomchai, R. (2013). The antimutagenic and antioxidant properties of *Spirogyra neglecta* (Hassall) Kutzing. Journals of medicinal plants research 7(34): 2494-2500.
Tipnee, S., Ramaraj, R. and Unpaprom, Y. (2015). Nutrition evaluation of edible freshwater green macroalga, *Spirogyra varians*. Emer Life Sci Res 1(2) : 1-7.
Traichaiyaporn, S., Kumano, S., Chainapong, T., Khuantrairong, T. and Waraegsiri, B. (2003). New record of freshwater red algae (Rhodophyta) in Thailand: *Batrachospermum cayennense* Montagne and Kützing, ScienceAsia 29: 85-87.
TraichaiyapornS., Kumano, S., Chainapong, T., Khuantrairong, T. and Waraegsiri, B. (2004). New record of freshwater red algae (Rhodophyta) in Thailand: *Batrachospermum mahlacense* Kumano et Boden-Kerby. ScienceAsia 30: 313-315.
Traichaiyaporn, S., Waraegsiri, B., Khuntrairong, T. and Kumano, S. (2007). Environmental factors and biodiversity of some freshwater red algae (Rhodophyta) in Thailand: Mae Hong Son province. Scientific Research Chulalongkorn University 6: 187-196.
Traichaiyaporn, S., Khuantrairong, T. and Kumano, S. (2008). *Thorea siamensis* sp.nov.(Thoreaceae: Rhodophyta) from Thailand. Natural History J. of Chulalongkorn University 8(1): 27-33.
Traichaiyaporn, S., Kumano, S. and Waraegsiri, B. (2012). A freshwater red algae, *Thorea clavata* Seto Ratnasabapathy, from Thailand with special reference to sexual reproductive organ. Maejo International Jorunal of Science and Technology 6(02): 216-223.
Vorrotnchaiphain, C. (2014). Integrated Trang Management from local stakholders's perpective. *p*. 8 . *In* Nexus dialogue on water infrastructure solutions. 17-19 March, Bangkok, Thailand.
Yucharoen, R., Srisuksomwong, P. and Tragoolpua,Y. (2015). Antibacterial and antioxidant activities of *Nostoc commune* Vaucher et Bornet et Flahault. Science &Technology RUMTT Journal 5(2): 35-48.