Journal of the Andaman Science Association Vol. 24(2):123-130 (2019)
ISSN 0970-4183, Printed in India

Phytochemical, Antioxidant and Antibacterial Activity of *Chromolaena odorata* from Andaman Islands, India

K. Saravanan[1*], T. Sivaramakrishnan[2], J. Praveenraj[1], R. Kiruba-Sankar[1], D. Suma[3], Varsha Devi[1] and Utpal Biswas[1]

[1]*ICAR- Central Island Agricultural Research Institute, Port Blair - 744105, Andaman and Nicobar Islands*
[2]*ICAR- Central Institute of Brackishwater Aquaculture, Chennai - 600028, Tamil Nadu*
[3]*ICAR- Central Institute of Fisheries Education, Mumbai - 400061, Maharashtra*
Corresponding author e-mail: sarocife@gmail.com

Abstract

The present study was aimed to evaluate the phytochemical, antioxidant and antibacterial activity of *Chromolaena odorata* (*Eupatorium odoratum*). *In-vitro* phytochemical and antioxidant properties of methanolic extract of *C. odorata* exhibited the maximum amount of total phenol (39.72 ± 5.25 mg GAE/ g), flavonoids (100.75 ± 1.75 mg RE/ g), DPPH radical scavenging activity (82.83 %), ABTS (23.18 mg/ g) and total antioxidant activity (75.42 mg/ g). Besides, the methanol and DMSO dissolved extracts of *C. odorata* revealed the considerable amount of *in-vitro* antibacterial activity against almost all the seven tested fish and human pathogenic bacteria. The highest amount of antibacterial activity with zone of inhibition of 21 mm was recorded against *Aeromonas hydrophila* and *Pseudomonas fluorescens*. The present study showed that the presence of potential active constituents in *C. odorata* may assist in combating the free radicals and also inhibits the growth of microbes.

Keywords: *Chromolaena odorata, Eupatorium odoratum,* phytochemical, antioxidant, antibacterial, Andaman Islands

Introduction

Chromolaena odorata (synonyms to *Eupatorium odoratum*) is a well known traditional medicinal herb belonging to the Family Asteraceae. Commonly called as siam weed, devil weed, French weed, Christmas bush, bitter bush, baby tea, Santa Maria and common floss flower (Prawiradiputra, 2007; Patel *et al.* 2010; Chakraborty *et al.* 2011; Vaisakh and Pandey, 2012). Even though it is an invasive species, possess various useful attributes such as antibacterial (Lavanya and Brahmaprakash, 2011; Ravikumar *et al.* 2011), anti-inflammatory (Owoyele *et al.* 2005; Ayyanar and Ignacimuthu, 2009; Vaisakh and Pandey, 2012), anti-malarial (Pisutthanan *et al.* 2005; Doss *et al.* 2011), anti-viral (Pisutthanan *et al.* 2005), anti-hepatotoxic (Alisi *et al.* 2011; Asomugha *et al.* 2014), anthelmintic (Vital and Rivera, 2009) and antioxidant (Venkata Raman *et al.* 2012) properties. It is also reported that the bioactive compounds present in *C. odorata* has the ability treat a variety of diseases (Vital and Rivera, 2009; Harlina *et al.* 2013).

In Andaman and Nicobar Islands, *C. odorata* is abundant and also used to cure various ailments by the local population including the indigenous tribes. The leaves of *C. odorata* are widely used by the indigenous tribes such as Nicobarese and Onges and they call it as 'Ureh bes' in Nicobari language which is used to treat against skin injuries, body ache, piles, centipede and snake bites (Chander *et al.* 2014; Chander and Vijayachari, 2018). Karen tribes call it as 'Sawpokwela' in their language and uses the leaves to cure fever, cough, skin injuries and breathing problems (Chander *et al.* 2015). In the wake of emerging antibiotic resistance and its subsequent transmission into food chain, the use of broad spectrum natural bioactive compounds without harmful side effects are highly encouraged for human and animal health management in the recent years. Studies on the phytochemical, antioxidant and antibacterial activity of *C. odorata* from Andaman and Nicobar Islands are scanty except the preliminary reports on its antimicrobial effect (Jai Sunder *et al.* 2012; Natheer *et al.* 2012). With this background, the present study was aimed at evaluation of phytochemical, antioxidant and antibacterial activity of *C. odorata* against fish and human pathogenic bacteria.

Materials and methods

(A). *In-vitro* phytochemical and antioxidant activity

Collection and preparation of sample

Fresh leaf samples of *C. odorata* were collected from ICAR-Central Island Agricultural Research Institute, South Andaman and shade dried at room temperature for 3-4 days. The dried leaves were homogenized with pestle and mortar and stored at 4 °C for further analysis.

Solvent extraction

One gram of *C. odorata* leaf powder was dissolved in 50 ml of 80 % methanol and incubated for 24 hours at room temperature. After incubation, the sample was centrifuged for 10 minutes and the supernatant was filtered into a fresh tube using Whattman no. 40 filter paper. The final crude methanol extract was used for phytochemical and antioxidant analysis.

Total phenolic content

Total phenolic content of the crude methanolic extract was determined using Folin-Ciocalteau (FC) assay as described by Singleton and Rossi (1965) with minor modification and the final results were expressed as Gallic acid equivalents (GAE)/ g of sample.

Total flavonoids

Total flavonoids content of the crude methanol extract was determined by spectrophotometric method of Zishen *et al*. (1999) with slight modification and the flavonoid content was expressed as mg Rutin equivalents per gram of dried extract (mg RE/ g).

Antioxidant assays

Crude methanolic extract of *C. odorata* was tested for its antioxidant activity. Different concentration of the extracted test samples (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ ml) were used for the assay. Standards were also taken in their respective concentrations.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The scavenging effect of crude methanol extract was determined according to the method of Farasat *et al*. (2013) with minor modification. The half-maximal inhibitory concentration (IC50) was calculated by the linear regression analysis and expressed as mean of determinations. Ascorbic acid was used as positive control.

ABTS (2, 2-azinobiz-3-ethylbenthiazoline-6-sulfonic acid) radical cation scavenging assay

Free radical scavenging activity of the methanolic extract was determined by following the method of Seenivasan *et al*. (2013) with minor modification. Trolox was used as standard.

Total antioxidant activity (TAA)

Total antioxidant activity of the crude methanolic extract obtained from *C. odorata* was determined according to Prieto *et al*. (1999) with slight modification. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

(B). *In-vitro* antibacterial activity

Preparation of extracts

In order to investigate *in-vitro* antibacterial activity, one gram of dried leaf powder of *C. odorata* was dissolved in 25 ml of 80 % methanol and kept for overnight incubation in an orbital shaking incubator. The sample was centrifuged at 4500 rpm for 10 minutes and then the supernatant was filtered with Whattman no. 1 filter paper. The filtrate was evaporated at 50 °C in hot air oven for complete evaporation of the solvent and based on the yield; the extract was diluted in 80 % methanol and DMSO separately, to obtain a concentration of 10 mg/ ml. The final solution was tested for its antibacterial activity against the selected bacteria.

Preparation of inoculum and plates for *in-vitro* antibacterial activity

Pure cultures of *Aeromonas hydrophilla* (ATCC 35654), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 4157), *Streptococcus pneumonia* (ATCC 49619), *Vibrio alginolyticus* (ATCC 17749), *Edwardsiella tarda* (ATCC 15947) and *Pseudomonas fluorescens* (ATCC 13525) were procured from Microbiologics, USA. The *in-vitro* antibacterial activity (Holder and Boyce, 1994). Bacterial strains were inoculated in nutrient broth and incubated at 37° C for 18-24 hours. Mueller Hinton agar plates were prepared and 50 µl of respective bacterial culture (10^7 cfu/ ml) was evenly spread throughout the plate using sterile glass L-rod spreader. Wells were made in each agar plate by using a sterile cork borer (diameter of 5.5 mm). Both methanol and DMSO dissolved extracts (50 µl) were loaded separately into the wells in duplicate. Fifty µl of streptomycin (10 mg/ ml) was used as positive control and the respective solvent alone of same volume was used as negative control. The plates were incubated at 37 °C and the zone of inhibition was measured after 24 hrs of incubation.

Statistical analysis

All the data were expressed as mean ± standard error in triplicates and the statistical analysis was executed using SPSS 16.0 software. One-way ANOVA and Duncan's multiple range tests were applied to check the significant difference among the mean and P value < 0.05 was regarded as significant.

Results and discussion

Andaman and Nicobar Islands are known for considerable amount of floral diversity and *Chromolaena odorata* is one among the species with ethno medicinal value and widely used to cure various ailments by the indigenous tribes (Chander and Vijayachari, 2018). In the present study, the leaves of *C. odorata* was selected for the investigation as it provides significantly higher percentage of yields than its other plant parts (Hanphakphoom *et al.* 2016). Methanol was employed for extraction as it is a high polar organic solvent reported to provide high extraction yield and high content of phytochemicals from the leaves of *C. odorata* (Stanley *et al.* 2014; Hanphakphoom *et al.* 2016).

The *in-vitro* phytochemical and antioxidant properties of *C. odorata* are provided in table 1, which depicts the total phenol (39.72 ± 5.25 mg GAE/ g), flavonoids (100.75 ± 1.75 mg RE/ g), DPPH radical scavenging activity (82.83 %), ABTS (23.18 mg/ g) and total antioxidant activity (75.42 mg/ g). The total phenol and flavonoid content obtained in the present study is much higher than the earlier study where the phenol content was reported to be in the range of 1.54 to 3.42 mg GAE/ g and flavonoid content to be 2.52 to 9.85 mg RE/ g for the leaf extract of *C. odorata* by using various solvents (Hanphakphoom *et al.* 2016). On the other hand, various studies revealed the presence of total phenolic contents and few studies could not detect the flavonoid contents and vice versa for *C. odorata* leaf extracts (Anyasor *et al.* 2011; Mondal *et al.* 2012; Danlami *et al.* 2013). But the present study revealed the higher amount of flavonoid than total phenol content as reported earlier (Hanphakphoom *et al.* 2016).

Table 1: *In-vitro* phytochemical and antioxidant activity of *Chromolaena odorata*. Data are represented as mean ± SE (n= 3).

Phytochemical & antioxidant activity	Results
Total Phenol	39.72 ± 5.25 mg GAE/ g
Flavonoid	100.75 ± 1.75 mg RE/ g
DPPH	82.83 %
TAA	75.42 mg/ g
ABTS	23.18 mg/ g

As far as DPPH radical scavenging activity of *C. odorata* is concerned, the result obtained in this present study is comparable with the earlier reports where it was found in the range of 5.65-87.93 % (Amatya and Tuladhar, 2011) and 24.68-61.78 % (Parameswari and Suriyavathana, 2012) for the ethanolic leaf extracts. Likewise, DPPH radical inhibition was observed to be 59.10 % and 52.13 % for the ethanolic and methanolic leaf extracts, respectively (Bhargava *et al.* 2013). Besides, the ABTS scavenging activity of ethanolic leaf extract of *C. odorata* was recorded as 29.92-63.34 % by Parameswari and Suriyavathana, (2012) and the total antioxidant activity of 10.39 µg/ ml ascorbic acid equivalent (AAE) was observed for the methanolic leaf extracts of *C. odorata* (Krishanti *et al.* 2010).

The differences in the phytochemical contents and its biological activity might be due to the type of extraction methods used, efficiency of the solvent used for extraction, ratio between the plant sample to solvent, different parts of the plant taken for analysis and the differences in their geographic location, climate, season and its growth phase (Maji *et al.* 2010; Kothari *et al.* 2012).

The fish pathogens *viz. Aeromonas hydrophila* causes haemorrhagic septicaemia and red pest disease; *Edwardsiella tarda* causes edwardsiellosis and emphysematous putrefactive disease; *Pseudomonas fluorescens* causes abdominal dropsy and fin rot disease and *Vibrio alginolyticus* causes vibriosis in fishes (Noga, 2010; Roberts, 2012). The human pathogenic bacteria such as *Escherichia coli* causes neonatal meningitis, enteric and systemic infections; *Staphylococcus aureus* causes endocarditis and staphylococcal food borne diseases and *Streptococcus pneumonia* causes pneumonia and meningitis in humans (Mitchell and Mitchell, 2010; Bachir and Abouni, 2015; Tong *et al.* 2015). All these pathogens are belonging to the gram negative group except, *Staphylococcus aureus* and *Streptococcus pneumonia* which falls under the gram positive category. In the recent past, ethno-medicine based treatment measures are getting prominence to control the above mentioned fish and human pathogenic infections in the light of emerging antimicrobial resistance.

In-vitro antibacterial activity of methanol and DMSO dissolved extracts of *C. odorata* was presented in table 2. Antibacterial activity of *C. odorata* was observed against all tested gram negative and gram positive bacteria except for the DMSO dissolved extract against *Escherichia coli*. Likewise, the highest amount of antibacterial activity with 21 mm of zone of inhibition was recorded against both *Aeromonas hydrophila* and *Pseudomonas fluorescens*. The zone of inhibition (mm) obtained in this present study against *E. coli, P. fluorescens, S. aureus* and *S. pneumonia* is much higher than the inhibition recorded in the earlier study for the same bacteria (Natheer *et al.* 2012). It was also reported that the ethanol and methanolic extracts of *Eupatorium odoratum* revealed antibacterial and anti-fungal activities against various tested organisms (Jai Sunder *et al.* 2012). Further, excellent antibacterial activity was exhibited by the extracts of *E. odoratum* against ten fish bacterial pathogens (particularly *Vibrio* sp.) isolated from the diseased ornamental fishes (Ravikumar *et al.* 2011). Likewise, methanolic extract of *C. odorata* showed the highest antibacterial activity against *V. harveyi* and also the artificial diet incorporated with bioactive compounds of *C. odorata* improves the survival rate of tiger prawn, *Penaeus monodon* (Harlina *et al.* 2015 & 2019). The reason behind the *in-vitro* growth inhibition of these microbes might be due to the presence of active constituents in the extracts which may act as alone or in combination (Natheer *et al.* 2012).

Free radicals (reactive oxygen species) are considered to be involved in many of the life threatening ailments. To combat the free radicals, plant based bioactive compounds (phyto-antioxidants) are considered as safe when compared to the synthetic antioxidants (Krishanti *et al.* 2010). Further, it is well documented that the antimicrobial compounds present in *C. odorata* such as flavonoids are helpful to inhibit the biosynthesis of cell wall of pathogens which in turn restrict its growth (Anyasor *et al.* 2011; Lavanya and Brahmaprakash, 2011). The above mentioned bioactive compounds present in *C. odorata* may be responsible for its curing activity against various ailments as evident by the earlier reports (Akinmoladun *et al.* 2007). As Andaman Islands

are known for its rich marine biodiversity, the present study elucidated that the terrestrial herbs present in these Islands such as *C. odorata* also possess the potentially considerable amount of phytochemical, antioxidant and antibacterial activity when compared to the earlier reports on seaweeds and mangroves (Sivaramakrishnan *et al.* 2017a,b & 2019). The present study revealed the potential of *C. odorata* to be used as a therapeutic against various ailments of humans as well as animals. However, further studies and in depth analysis on various compounds and its properties need to be elucidated towards its potential industrial application on a large scale for the benefit of animal and human mankind.

Table 2: *In-vitro* antibacterial activity of methanol and DMSO dissolved extracts of *Chromolaena odorata*

Bacteria	Zone of inhibition (mm)					
	Methanol extract (50 µl) 10 mg/ ml	Positive control (50 µl) (Streptomycin) 10 mg/ ml	Negative control (50 µl) (Methanol)	DMSO extract (50 µl) 10 mg/ ml	Positive control (50 µl) (Streptomycin) 10 mg/ ml	Negative control (50 µl) (DMSO)
Aeromonas hydrophila	18	42	0	21	30	0
Escherichia coli	15	24	0	0	21	0
Vibrio alginolyticus	6	24	0	14	27	0
Edwardsiella tarda	14.5	26	0	15.5	24	0
Pseudomonas fluorescens	21	26	0	9.5	23	0
Staphylococcus aureus	11.5	18	0	10	18	0
Streptococcus pneumonia	10	29	0	14.5	27	0

Acknowledgements

The authors are grateful to the Director, ICAR-Central Island Agricultural Research Institute, Port Blair for providing the necessary facilities to carry out the research work.

References

Akinmoladun, A.C., Ibukun, E.C. & DanOloge, I.A. (2007). Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. *Scientific Research and Essays*, 2: 191-194.

Alisi, C.S., Onyeze, G.O.C., Ojiako, O.A. & Osuagwu, C.G. (2011). Evaluation of the protective potential of *Chromolaena odorata* Linn. extract on carbon tetrachloride-induced oxidative liver damage. *International Journal of Biochemistry Research and Review*, 1(3): 69-81.

Amatya, S. & Tuladhar, S.M. (2011). *In vitro* Antioxidant Activity of Extracts from *Eupatorium odoratum* L. *Research Journal of Medicinal Plants,* 5: 79-84.

Anyasor, G.N., Aina, D.A., Olushola, M. & Aniyikaye, A.F. (2011). Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena odorata*. *Annals of Biological Research*, 2(2): 441-451.

Asomugha, R.H., Okafor, P.N., Ijeh, I.I., Orisakwe, O.E. & Asomugha, A.L. (2014). Hepatic effects of aqueous extract of *Chromolaena odorata* in male Wistar albino rats. *Pharmacology Online*, 1: 127-136.

Ayyanar, M. & Ignacimuthu, S. (2009). Herbal medicines for wound healing among tribal people of Southern India: Ethnobotanical and scientific evidences. *International Journal of Applied Research in Natural Products,* 2(3): 29-42.

Bachir, R.G. & Abouni, B. (2015). *Escherichia coli* and *Staphylococcus aureus* most common source of infection. pp. 637-648. In: The battle against microbial pathogens: Basic Science, Technological Advances and Educational Programs, (Eds.) Méndez-Vilas, A., Formatex Research Center, Spain.

Bhargava, D., Mondal, C.K., Shivapuri, J.N., Mondal, S. & Kar, S. (2013). Antioxidant properties of the leaves of *Chromolaena odorata* Linn. *Journal of Institute of Medicine,* 35(1): 53-56.

Chakraborty, A.K., Rambhade, S. & Patil, U.K. (2011). *Chromolaena odorata* (L.): an overview. *Journal of Pharmacy Research*, 4(3): 573-576.

Chander, P.M. & Vijayachari, P. (2018). Ethnomedicinal knowledge among the tribes of the Little Andaman Island, Andaman and Nicobar Islands, India. *Pharmacognosy Magazine,* 14: S488-93.

Chander, P.M., Kartick, C. & Vijayachari, P. (2015). Ethnomedicinal knowledge among Karens of Andaman & Nicobar Islands, India. *Journal of Ethnopharmacology*, 162: 127-133.

Chander, P.M., Kartick, C., Gangadhar, J. & Vijayachari, P. (2014). Ethno medicine and healthcare practices among Nicobarese of Car Nicobar - An indigenous tribe of Andaman and Nicobar Islands. *Journal of Ethnopharmacology,* 158: 18–24.

Danlami, U., Adebisi, F.A., David, B.M., Lawal, D.R. & Galadanchi, K.M. (2013). Proximate and phytochemical analyses of the hexane, ethyl acetate and ethanol extracts of *Chromolaena odoratum* (Linn.) leaves. *Asian Journal of Pharmaceutical & Biological Research*, 3(1): 34-35.

Doss, A., Parivuguna, V., Vijayasanthi, M. & Sruthi, S. (2011). Antibacterial evaluation and phytochemical analysis of certain medicinal plants, Western Ghats, Coimbatore. *Journal of Research in Biology,* 1: 24-29.

Farasat, M., Khavari-Nejad, R.A., Nabavi, S.M. & Namjooyan, F. (2013). Antioxidant properties of two edible green seaweeds from northern coasts of the Persian Gulf. *Jundishapur Journal of Natural Pharmaceutical Products,* 8: 47–52.

Hanphakphoom, S., Thophon, S., Waranusantigu, P., Kangwanrangsan, N. & Krajangsang, S. (2016). Antimicrobial activity of *Chromolaena odorata* extracts against bacterial human skin infections. *Modern Applied Science,* 10(2): 159-171.

Harlina, H., Prajitno, A., Suprayitno, E. & Nursyam, H. (2013). The identification of chemical compound and antibacterial activity test of kopasanda (*Chromolaena odorata* L.) leaf extract again vibriosis-causing *Vibrio harveyi* (mr 275 rif) on tiger shrimp. *Aquatic Science and Technology*, 1: 15-29.

Harlina, H., Prajitno, A., Suprayitno, E., Nursyam, H. & Rosmiati. (2015). Potential study of Kopasanda (*Chromolaena odorata* L.) leaves as antibacterial against *Vibrio harveyi*, disease causative agent of tiger shrimp (*Penaeus monodon* Fabricius) post larvae. *Journal of Aquaculture Research and Development,* 6(10): 372. doi:10.4172/2155-9546.1000372.

Harlina, H., Rosmiati, Jafar, S., Sukmawati, Nurhidaya & Kamaruddin. (2019). Effect of *Chromolaena odorata* as bioactive compound in artificial diet on survival rate of tiger prawn *Penaeus monodon. IOP Conference Series: Earth and Environmental Science,* 253 012015. doi:10.1088/1755-1315/253/1/012015.

Holder, I.A. & Boyce, S.T. (1994). Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns,* 20: 426-429.

Jai Sunder, Kundu, A., Jeyakumar, S. & De, A.K. (2012). Antimicrobial activities of *Eupatorium odoratum* leaves. *Indian Veterinary Journal*, 89(1): 24-25.

Kothari, V., Gupta, A. & Naraniwal, M. (2012). Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds. *Journal of Natural Remedies*, 12(2): 162-173.

Krishanti, M.P., Rathinam, X., Kasi, M., Ayyalu, D., Surash, R., Sadasivam, K. & Subramaniam, S. (2010). A comparative study on the antioxidant activity of methanolic leaf extracts of *Ficus religiosa* L, *Chromolaena odorata* (L.) King & Robinson, *Cynodon dactylon* (L.) Pers. and *Tridax procumbens* L. *Asian Pacific Journal of Tropical Medicine,* 348-350.

Lavanya, G. & Brahmaprakash, G.P. (2011). Phytochemical screening and antimicrobial activity of compounds from selected medicinal and aromatic plants. *International Journal of Science and Nature*, 2(2): 287-291.

Maji, S., Dandapat, P., Ojha, D., Maity, C., Halder, S. K., Das Mohapatra, P. K. & Mondal, K.C. (2010). *In vitro* antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. *Journal of Phytology*, *2*(4): 57-64.

Mitchell, A.M. & Mitchell, T.J. (2010). *Streptococcus pneumoniae:* virulence factors and variation. *Clinical Microbiology and Infection,* 16: 411–418.

Mondal, K.C., Bhargava, D., Shivapuri, J.N. & Kar, S. (2012). In vitro antigonorrhoeal activity and extraction of chemical constituents from the leaves of *Chromolaena odorata* (Lin.) locally known as BANMARA. *International Journal of Chemical and Analytical Science*, 3(7): 1487-1495.

Natheer, S.E., Sekar, C., Amutharaj, P., Rahman, M.S.A. & Khan, K.F. (2012). Evaluation of antibacterial activity of *Morinda citrifolia, Vitex trifolia* and *Chromolaena odorata*. *African Journal of Pharmacy and Pharmacology*, 6: 783-788.

Noga, E.J. (2010). Fish disease: diagnosis and treatment, 2nd edition. Mosby-year Book, Wiley-Blackwell Publishing Inc., USA. 519 p.

Owoyele, V.B., Adedij, J.O. & Soladoy, A.O. (2005). Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata*. *Inflammopharmacology,* 13(5- 6): 479-484.

Parameswari, G. & Suriyavathana, M. (2012). *In vitro* Antioxidant Activity of *Chromolaena odorata* (L.) King & Robinson. *International Research Journal of Pharmacy,* 3(11): 187-192.

Patel, J., Kumar, G.S., Qureshi, M.S. & Jena, P.K. (2010). Anthelmintic activity of Ethanolic extract of whole plant of *Eupatorium Odoratum. L. International Journal of Phytomedicine,* 2: 127-132.

Pisutthanan, N., Liawruangrath, B., Liawruangrath, S., Baramee, A., Apisariyakul, A., Korth, J. & Bremner, J.B. (2005). Constituents of the essential oil from aerial parts of *Chromolaena odorata* from Thailand. *Natural Product Research*, 20(6): 636-640.

Prawiradiputra, B.R. (2007). Ki Rinyuh (*Chromolaena odorata* (L) R M King dan H Robinson: Gulma padang rumput yang merugikan. *Wartazoa,* 17(1).

Prieto, P., Pineda, M. & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry,* 269: 337-341.

Ravikumar, S., Anitha Anandha Gracelin, N., Palani Selvan, G. & Kalaiarasi, A. (2011). In vitro antibacterial activity of coastal medicinal plants against isolated bacterial fish pathogens. *International Journal of Pharmaceutical Research and Development*, 3(4): 109-116.

Roberts, R.J. (2012). Fish Pathology, 4th edition. Blackwell Publishing Ltd., UK. 590 p.

Seenivasan, R., Rekha, M., Indu, H. & Geetha, S. (2013). Antibacterial activity and phytochemical analysis of selected seaweeds from Mandapam coast. *Indian Journal of Pharmaceutical Science,* 2(10): 159-169.

Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagent. *American Journal of Enology and Viticulture,* 16: 144-158.

Sivaramakrishnan, T., Biswas, L., Shalini, B., Saravanan, K., Kiruba Sankar, R., Goutham Bharathi, M.P. & Dam

Roy, S. (2017a). Analysis of proximate composition and in-vitro antibacterial activity of selected green seaweeds from South Andaman coast of India. *International Journal of Current Microbiology and Applied Sciences,* 6(12): 1739-1749.

Sivaramakrishnan, T., Swain, S., Saravanan, K., Kiruba Sankar, R., Dam Roy, S., Biswas, L. & Shalini, B. (2017b). In-vitro antioxidant and free radical scavenging activity and chemometric approach to reveal their variability in green macroalgae from South Andaman coast of India. *Turkish Journal of Fisheries and Aquatic Sciences,* 17: 639-648.

Sivaramakrishnan, T., Swain, S., Saravanan, K., Rajendran, K.S. & Dam Roy, S. (2019). *In vitro* antioxidant, free radical scavenging activity and chemometric approach to reveal variability in different solvent extracts from selected mangroves of Andaman and Nicobar Islands, India. *Journal of Coastal Research,* Special Issue 86: 263-269.

Stanley, M.C., Ifeanyi, O.E., Nwakaego, C.C. & Esther, I.O. (2014). Antimicrobial effects of *Chromolaena odorata* on some human pathogens. *International Journal of Current Microbiology and Applied Sciences*, 3(3): 1006-1012.

Tong, S.Y.C., Davis, J.S., Eichenberger, E., Holland, T.L. & Fowler, V.G.Jr. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews,* 28(3): 603-61.

Vaisakh, M.N. & Pandey, A. (2012). Pharmacognostic study of leaves of Chromolaena odorata UNM. *International Journal of Pharmaceutical Research and Development*, 4(2): 33-37.

Venkata Raman, B., Samuel, L., Pardha Saradhi, M., Narashimha Rao, B., Naga Vamsi ar, M. & Radhakrishnan, T.M. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian Journal of Pharmaceutical and Clinical Research*, 5(2): 99-106.

Vital, P.G. & Rivera, W.L. (2009). Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. extracts. *Journal of Medicinal Plants Research*, 3(7): 511-518.

Zishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging affects on superoxide radicals. *Food Chemistry,* 64: 555-559.

Received : 10th June 2019 Accepted : 2nd November 2019