INT. J. BIOL. BIOTECH., 21 (2): 175-180, 2024.

COMPARATIVE ANALYSIS OF MOSQUITO REPELLENCY POTENTIAL IN THREE BROWN SEAWEEDS COLLECTED FROM THE COAST OF KARACHI, PAKISTAN

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ABSTRACT

Mosquito borne diseases pose significant public health threat, especially in tropical region. Being rich in primary and secondary metabolites, seaweeds possess mosquito-repelling properties among other well-known pharmacological properties. This study focused on mosquito repelling and mosquitocidal properties of three seaweeds- *Iyengaria stellata, Colpomenia sinuosa,* and *Sargassum wightii.* Results showed that crude extracts of *S. wightii* had higher mosquito repellency, while the fraction of n-Hexane repelled mosquitoes for the longest period of time, which was 3.5 hours. However *C. sinuosa* was found to be the least repellent among three seaweeds.

Key-words: Mosquito repellency, *Sargassum wightii*, *Iyengaria stellata*, *Colpomenia sinuosa*

INTRODUCTION

Mosquito-borne diseases, which account for roughly 17% of all infectious diseases, cause approximately one million fatalities throughout the world annually (Lawal *et al*, 2012; Ranasinghe *et al*, 2016). WHO has also reported that about 390 million people are annually affected by dengue fever, an infection caused by the mosquito vector *Aedes aegypti* (Ahmed *et al*, 2019; Jangir and Prasad, 2022). The fact that there is no particular treatment for dengue fever, and mosquito-borne diseases, in general, are becoming more prevalent, leads to advancement in precautionary measures against mosquito bites (Hira *et al*., 2010).

Although a number of natural and synthetic mosquito repellents are present, synthetic mosquito repellents are harmful to the environment including humans. The chemicals used in synthetic mosquito repellent affect non-target organisms, as they are often discharged in the oceans, from where they enter the entire food web (Yu *et al*., 2015). In humans, these repellents not only cause skin allergies, but are also responsible for affecting liver and kidneys, cause respiratory disorders including asthma and pneumonia, and irritation in the eyes (Hogarh *et al*., 2018; Andini *et al*., 2022; Islam *et al.,* 2022).

Natural products are relatively safer and more environment-friendly, and can be formulated in the form of mosquito repellents (Asadollahi *et al.,* 2019), but some studies suggest that mosquitos have started developing resistance against them (Esmaili *et al*, 2021). As a result, in the past few years, scientists have developed an interest in employing seaweeds for their mosquito-repelling ability. Seaweeds are abundant in primary and secondary metabolites, including minerals, vitamins, dietary fibers, polyphenols, flavonoids, terpenes, polysaccharides, fatty acids and saponins, *etc*. Due to presence of these beneficial compounds, antidepressant, anticancer, fat-lowering, antihypertensive, antidiabetic, antibacterial, antifungal, antiviral,nematicidal and insecticidal properties of seaweeds have already been reported (Ahmed *et al*., 2012; Anis *et al*., 2017; Rosa *et al.,* 2019; Al Monla *et al*., 2020). Therefore over the years, seaweeds have gained interest in being tested against mosquito vectors (Rizvi and Shameel, 2005; Hira *et al*., 2010; Yu *et al*, 2015).

Pakistan is home to a coastline of 886km, which is divided into two main regions, Sindh coastline and Baluchistan coastline. Sindh has a coastline that stretches for around 100km, and the region is home to a diverse range of seaweeds (Shameel and Tanaka, 1992). This research focused on the mosquito-repelling properties of three brown seaweeds; namely *Iyengaria stellata* (Børgesen) Børgesen, 1939, *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier and *Sargassum wightii* Greville ex J.Agardh. The main objectives were to identify and compare the three seaweed's ability to repel adult mosquitoes, the impact of various solvents on the seaweed's mosquito repellency, and the mortality rate of mosquitoes in response to seaweed extracts. Although there have been reports of seaweed's tendency to repel mosquito larvae(Vimaladevi *et al*., 2012; Bibi *et al*., 2020; Dhanasundaram *et al*., 2020), this research targeted adult-stage mosquitoes, making this research a significant innovation in studies of mosquito repellency while employing seaweeds as a natural repellent.

MATERIALS AND METHODS

Collection site:

Buleji is a well-bound protected area, and possesses rocky ledges facing sea as well as sandy area. The ledges are about 3-4 meters long, and possess rock pools along with small bay regions between whereas the sandy area is spread on 4-5 acres (Hayee-Memon and Shameel, 1996).

Sample Collection and Processing:

With an elevation of about 16 meters above sea level, Buleji (24º 50'20.41'' N, 66º 49'24.15'' E) (Ahmed *et al.,* 2020) is situated close to French beach, Karachi **(Fig. 1)**. Seaweeds *Iyengaria stellata* (Børgesen) Børgesen, 1939, *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier and *Sargassum wightii* Greville ex J.Agardh were collected from bay regions and sandy areas of Buleji, where they were found to be firmly attached to the substratum by their holdfasts. The collected samples werewashed to remove debris and epiphytes. A few samples were preserved in 4% formalin and on herbarium sheets for identification purposes, while large quantities of collected samples were shade-dried, crushed into powder form and then stored until further use.

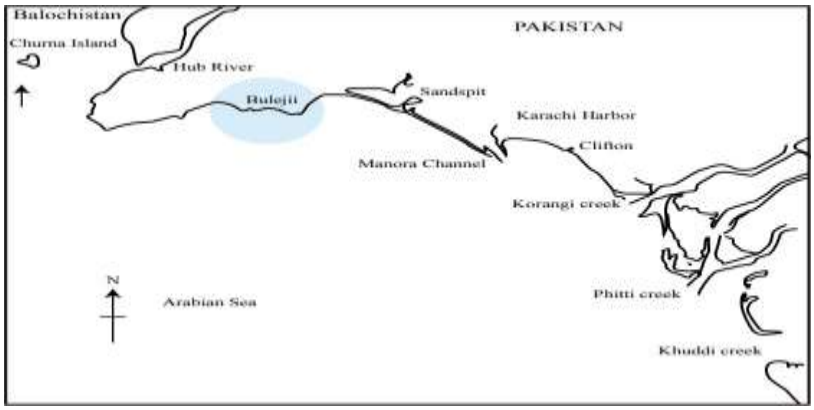

Fig. 1. Karachi coastline showing site of seaweed collection.

Extraction and Fractionation:

In accordance with the ratios established by Bambang *et al*. (2013), seaweeds were immersed separately in methanol, ethanol, ethyl acetate and n-Hexane. Following a 15-day time period, the extracts were filtered, and the seaweed residue were re-soaked with respective solvents. The procedure was carried out three times. The extracts were collected each time in respective bottles and processed through rotary evaporator at 50-60°C to obtain crude extracts. These crude extracts were used for further tests. The procedure was carried out as a modified version of Rizvi and Shameel (2004).

Mosquito Repellency Test:

Crude seaweed extracts were evaluated for their ability to repel adult *Aedes aegypti* mosquitoes by using the filter paper method described by Rizvi and Shameel (2004), and the hand in-cage method as described by Tariq and Qadri (2001). The mosquitoes were cultured in PCSIR Complex, Karachi. Repellency in terms of percentage, mortality rate, and complete protectiontime (CPT) were recorded. The mosquito repellency (%) was calculated by following equation:

$$Repellency\ \%: [\frac{Control - \{Treatment\}}{Control}] * 100$$

RESULTS AND DISCUSSION

Comparison between Repellency and Mortality Rate:

All the seaweeds tested, *i.e., C. sinuosa*, *I. stellata* and *S. wightii* demonstrated effective mosquito repellency as well as mortality, but the latter was less common than the former. The response of seaweeds against mosquitoes was *S. wightii* > *I. stellata* > *C. sinuosa* (Table 1). These findings are corroborated by research conducted by Suganya *et al.,* (2019), who found that ethanolic extracts of *S. wightii* and *Halimeda gracillis* were repellent to

third-star larvae of three different mosquito species. In addition, Yu *et al,* (2015), demonstrated that *Bryopsis pennata* effectively repels larvae and female oviposition of *Aedes aegypti*. In our study, *I. stellata* exhibited higher repellency and mortality than *C. sinuosa*. This outcome can be attributed to high antioxidant content of *I. stellata* because higher antioxidant concentrations are associated with higher levels of insecticidal activity (Ahmad *et al.*, 2013; Sivagnanam *et al.*, 2015).

Table 1. Mosquito Repelling and Mortality Properties of Seaweeds.

Sample	Repellency	Mortality
Iyengaria stellata	5 ± 0.577	2 ± 0.333
Colpomenia sinuosa	3 ± 0.577	1 ± 0.333
Sargassum wightii	6.33 ± 0.333	3 ± 0.57

Repellency (%) of Three Seaweeds:

Previous studies conducted by Achary *et al,* (2014), showed that extracts of *S. wightii* show mosquito repellency against larvae of *Aedes aegypti* for up to 72 hours. This confirms results of our study where *Sargassum wightii* displayed highest mosquito repellency, followed by *Iyengaria stellata*, while *Colpomenia sinuosa* showed the least mosquito repellency rate.

In our study, it was also observed that *S. wightii* had maximum mosquito repellency which was 28% when treated against adult mosquitoes (Table 2). Previously, Yogarajalakshmi *et al*, (2020) showed prominent mosquito repellency, and 98% and 97% mortality against III and IV star larvae of mosquitoes when treated with ethanolic extracts of red algae *Champia parvula*. It is possible that the adult stage of mosquitoes in our study contributed to the lower repellency rate. Our study focused on the ability of seaweeds to deter adult mosquitoes, as opposed to the usual studies on ovicidal and larvicidal effects of seaweeds against mosquitoes (Yu *et al.*, 2015).

Table 2. Repellency Rate (%) of Brown Seaweeds.

Sr. No	Seaweed Used	Dose (mL)	Repellency (%)
1	*Iyengaria stellata*	0.5	23
2	*Colpomenia sinuosa*	0.5	18
3	*Sargassum wightii*	0.5	28

Rate of Repellency (%) in Each Fraction:

Each seaweed was treated with four different solvents - Methanol, Ethanol, Ethyl Acetate and n- Hexane - for mosquito-repelling properties. In the case of seaweed *I. stellata,* the fraction of ethyl acetate displayed highest mosquito repelling property, closely followed by the fraction of n-Hexane. In the past, Kumar *et al.* (2017) reported that n-Hexane can be used to extract oils from natural resources. This appears to be a potential explanation for the higher mosquito repellency in the n-Hexane fraction of *I. stellata* in our study, as Usmanghani *et al*. (1987) reported the presence of natural oils in *I. stellata* and Ramar *et al.* (2014) reported that essential oils obtained from natural resources can be used as mosquito repellents. It was observed that *C. sinuosa* was the least repellent seaweed in all fractions. Previous study conducted by Minatel *et al.* (2017) suggests that antioxidant activity is dependent on the number of phenolic compounds present in the organism, and Ahmad *et al*. (2013) suggested that insecticidal activity is related to the antioxidant property of the organism, therefore it is possible that *C. sinuosa* was less effective in repelling mosquitoes because it had least amount of phenolic compounds among all three seaweeds tested. It was also observed in this study that *S. wightii* showed maximum mosquito repellency in methanol fraction (**Fig. 2**). This result is supported by study conducted by Sadati *et al,* (2011), which suggested that in brown seaweed *S. swartzaii*, a fraction of MeOH- H_2O, shows higher antioxidant activity and can be used for pharmacological effects.

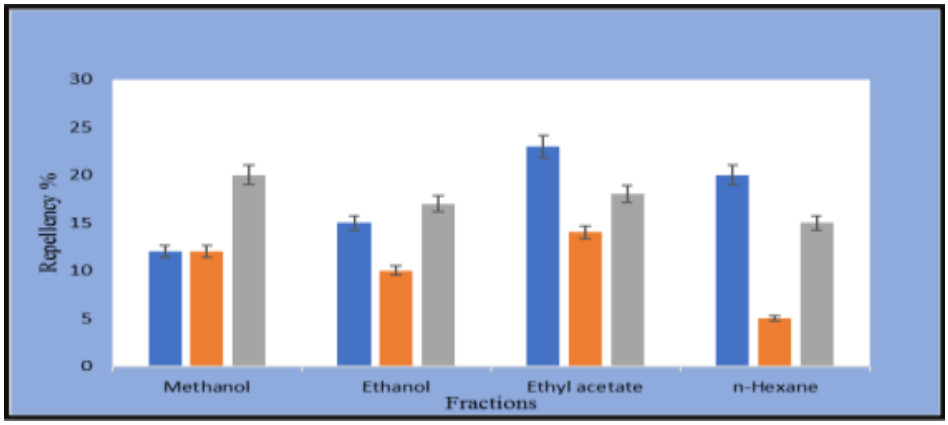

Fig 2. Repellency (%) of each fraction of three seaweeds.

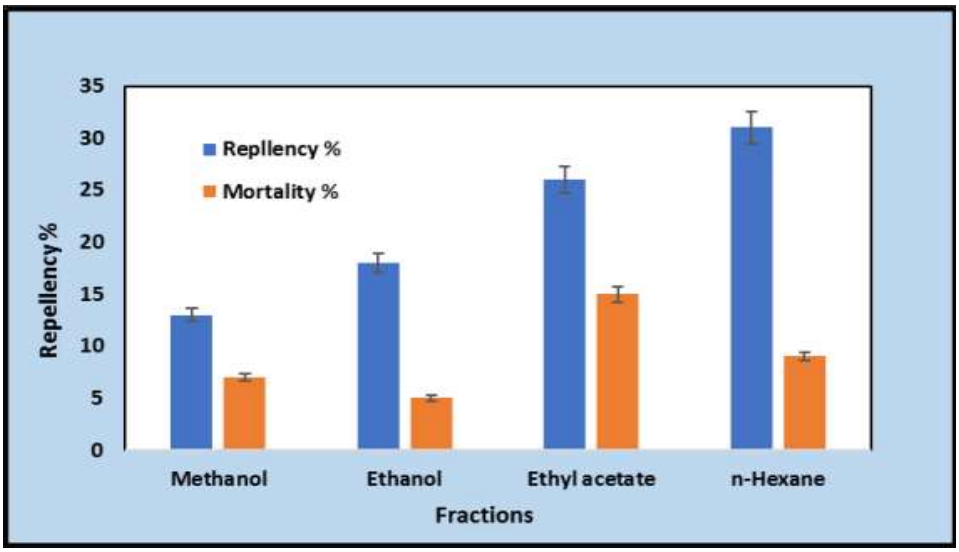

Fig 3. ∑ of each fraction of three seaweeds.

Combined Effects of Fractions on Repellency (%):

It was observed during mosquito repelling testing that mixing of fraction-based extracts of the three seaweeds tested considerably alters their impact on mosquito repellency and mortality. When combined, the fraction of n-Hexane demonstrated the highest mosquito repelling property, whilst the fraction of ethyl acetate displayed highest mortality rate. Among all four fractions tested, ethanol based fraction had the lowest mortality rate of mosquitoes, while methanol fraction had lowest mosquito repellency rate (**Fig. 3**). It has been previously demonstrated by Ramanathan *et al*, (2022) that fraction of ethyl acetate obtained from marine grass *Cymodocea serrulata* has a positive response in repelling mosquitoes, and can be employed as a potential control against the mosquito vector *Culex quinquefasciatus*. In the past, Elango *et al*., (2009) hasalso reported that extract obtained with the help of ethyl acetate from the plant *Cocculus hirsutus* (L.) Diels can keep mosquitoes away for two hours or 120 minutes whereas in our study, it was observed that while n-Hexane and ethyl acetate repelled mosquitoes for 3.5 and 3 hours respectively, methanol and ethanol fractions showed repellency for only 1.5 hours.

CONCLUSION

In this study three brown seaweeds, *i.e., I. stellata, C. sinuosa* and *S. wightii* were tested for their mosquito-repelling properties. All three seaweeds showed mosquito repellency as well as mosquitocidal properties, although the rate of mortality was lower as compared to the rate of repellency. Additionally, it was discovered that the

fraction tested affected both the rate as wellas the time duration of mosquito repellency. While the fraction of n-Hexane had the longest time duration of mosquito repellency, the highest repellency rate was observed in the fraction of ethyl acetate. Although this research reports mosquito repellency against adult mosquitoes for the first time from this region, this is still a baseline study, and further research is still required to determine the exact concentrations and minimum lethal doses of these fractions as mosquito repellents.

ACKNOWLEDGEMENTS

The authors acknowledge Prof. Dr. Ehtesham-ul-Haque (Late), Director of M.A.H. Qadri Biological Research Centre, University of Karachi for providing laboratory facilities for relevant experiments, and Dr. M. Tariq Rajput, Department of Zoology, University of Karachi for his guidance in hand, in cage experiment of mosquito repellency.

REFERENCES

Achary, A., K. Muthalagu and M.S. Guru (2014). Identification of Phytochemicals from *Sargassum wightii* against *Aedes aegypti*. *International Journal of Pharmaceutical Sciences, Review and Research*, 29 (1): 314-319.

Ahmad, M., F. Saeed and N. Jahan (2013). Evaluation of insecticidal and anti-oxidant activity of selected medicinal plants. *Journal of Pharmacognosy and Phytochemistry*, 2 (3): 153-158.

Ahmed, Q., A.S. Thandar and Q.M. Ali (2020). *Holothuria (Lessonothuria) insignis* Ludwig, 1875 (formally resurrected from synonymy of *H. pardalis* Selenka, 1867) and *Holothuria (Lessonothuria) lineata* Ludwig, 1875—new additions to the sea cucumber fauna of Pakistan, with a key to the subgenus *Lessonothuria* Deichmann (Echinodermata:Holothuroidea). *Zootaxa*, 4767(2), 307-318.

Ahmed, S., M. Hasan, M. Ali and I. Azhar (2012). Antiemetic activity of *Iyengaria stellata* and *Valoniopsis pachynema* in chicks. *International Journal of Phycology and Phycochemistry*, 8 (2): 127-132.

Ahmed, T., M.Z. Hyder, I. Liaqat and M. Scholz (2019). Climatic conditions: conventional and nanotechnology-based methods for the control of mosquito vectors causing human health issues. *International journal of environmental research and public health*, 16 (17): 3165-3189.

Al Monla, R.M., Z.T. Dassouki, H. Gali-Muhtasib and H.R. Mawlawi (2020). Chemical analysis and biological potentials of extracts from *Colpomenia sinuosa*. *Pharmacognosy Research*, 12 (3): 272-277.

Andini, A., M. Hasanah, S.N. Azizah, A.R. Rosyadahan, D. Rimasari, W. Triapadma, F. Ayu, and A. Syafiuddin (2022). The effect of insect repellent exposure on leukocyte profile and histopathologic findings in lungs. *Biointerface Research in Applied Chemistry*, 12 (6): 7796-803.

Anis, M., S. Ahmed and M.M. Hasan (2017). Algae as nutrition, medicine and cosmetic: The forgotten history, present status and future trends. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6 (6): 1934-1959.

Asadollahi, A., M. Khoobdel, A. Zahraei-Ramazani, S. Azarmi and S.H. Mosawi (2019). Effectiveness of plant-based repellents against different Anopheles species: a systematic review. *Malaria Journal*, 18 (1): 1-20.

Bambang, B.S., S. Kumalaningsih and W.H. Susinggih (2013). Polyphenol content and antioxidant activities of crude extract from brown algae by various solvents. *Journal of Life Science and Biomedicine*, 3: 439-443.

Bibi, R., R.M. Tariq, and M. Rasheed, (2020). Toxic assessment, growth disrupting and neurotoxic effects of red seaweeds' botanicals against the dengue vector mosquito *Aedes aegypti* L. *Ecotoxicology and Environmental Safety*, 195: 110451.

Dhanasundaram, S., A. Aravinth, P. Perumal, V. Amutha, R. Rajaram and P. Santhanam (2022). Mosquito-pupicidal activity of the extracts of seaweeds, *Sargassum wightii*, and *Gelidiella acerosa* against the dengue vector, *Aedes aegypti*. *Biomass Conversion and Biorefinery*, 1-8.

Elango, G., A. Bagavan, C. Kamaraj, A. Abduz Zahir and A. Abdul Rahuman (2009). Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). *Parasitology Research*, 105: 1567-1576.

Esmaili, F., A. Sanei-Dehkordi, F. Amoozegar and M. Osanloo (2021). A review on the use of essential oil-based nanoformulations in control of mosquitoes. *Biointerface Research in Applied Chemistry*, 11 (5): 12516-12529.

Hayee-Memon, A., and M. Shameel, (1996). A taxonomic study of some red algae commonly growing on the coast of Karachi. *Pakistan Journal of Marine Sciences*, 5 (2): 113-137.

Hira, V.S., R.M. Tariq, J. Ara and S. Ehteshamul-Haque (2010). Larvicidal activity of marine macro-algae from Karachi coast against dengue virus vector mosquito, the *Aedes aegypti* L. *Pakistan Journal of Entomology*, 28 (2): 143-148.

Hogarh, J.N., T.P. Agyekum, C.K. Bempah, E.D. Owusu-Ansah, S.W. Avicor, G.A. Awandare, J.N. Fobil and K. Obiri-Danso (2018). Environmental health risks and benefitsof the use of mosquito coils as malaria prevention and control strategy. *Malaria Journal*, 17: 1-12.

Islam, M., M.Z. Haider and S.F.B. Halim (2022). Health hazard of using mosquito repellent in Khulna city, Bangladesh. *Journal of Economics and Development*, 24 (1): 65-79.

Jangir, P.K and A. Prasad (2022). Spatial distribution of insecticide resistance and susceptibility in *Aedes aegypti* and *Aedes albopictus* in India. *International Journal of Tropical Insect Science*, 42 (2): 1019-1044.

Kumar, S.J., S.R. Prasad, R. Banerjee, D.K. Agarwal, K.S. Kulkarni and K.V. Ramesh (2017). Green solvents and technologies for oil extraction from oil seeds. *Chemistry Central Journal*, 11 (1): 1-7.

Lawal, H.O., G.O. Adewuyi, A.B. Fawehinmi, A.O. Adeogun and S.O. Etatuvie (2012). Bioassay of herbal mosquito repellent formulated from the essential oil of plants. *Journal of Natural Products*, 5: 109-115.

Minatel, I. O., C.V. Borges, M.I. Ferreira, H.A.G. Gomez, C.-Y.O. Chen and G.P.P. Lima (2017). Phenolic Compounds: Functional Properties, Impact of Processing and Bioavailability. *InTechOpen*, 8: 1-24.

Ramanathan, M., M. Sukumaran, M. Narayanan, N. Devarajan, A. Chinnathambi, S.A. Alharbi, N.T.L. Chi, M. Saravanan and K. Brindhadevi (2022). Larvicidal and pupicidal activity of crude ethyl acetate extract fraction-7a of *Cymodocea serrulata* on *Culex quinquefasciatus*. *Process Biochemistry*, 122: 166-171.

Ramar, M., S. Ignacimuthu and M.G. Paulraj (2014). Mosquito knock-down and adulticidal activities of essential oils by vaporizer, impregnated filter paper and aerosol methods. *International Journal of Mosquito Research*, 1 (3): 26-32.

Ranasinghe, M.S.N., L. Arambewela and S. Samarasinghe (2016). Development of herbal mosquito repellent formulations. *International journal of pharmaceutical Sciencesand Research*, 7 (9): 3643-3648.

Rizvi, M.A. and M. Shameel (2004). Biological activity and elementology of benthic algae from Karachi coast. *Pakistan Journal of Botany*, 35 (5; SPI): 717-730.

Rizvi, M.A. and M. Shameel (2005). Pharmaceutical biology of seaweeds from the Karachi coast of Pakistan. *Pharmaceutical biology*, 43 (2): 97-107.

Rosa, G.P., W.R. Tavares, P.M. Sousa, A.K. Pagès, A.M. Seca, and D.C. Pinto (2019). Seaweed secondary metabolites with beneficial health effects: An overview of successes in in vivo studies and clinical trials. *Marine drugs*, 18 (1): 8-42.

Sadati, N., M. Khanavi, A. Mahrokh, S.M.B. Nabavi, J. Sohrabipour and A.Hadjiakhoondi (2011). Comparison of antioxidant activity and phenolic contents of some Persian Gulf marine algae. اطنیگ یپ ژوش یع لم ن ص ل زاهج ی دارو, 10 (37): 73-79.

Shameel, M. and J. Tanaka (1992): A preliminary check-list of marine algae from coast and inshore waters of Pakistan. In: Cryptogamic Flora of Pakistan. Vol. 1 (Eds.) T. Nakaike and S. Malik. Nat. Sci. Mus., Tokyo, p. 1-64.

Sivagnanam, S.P., S. Yin, J.H. Choi, Y.B. Park, H.C. Woo and B.S. Chun (2015). Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO_2 extraction. *Marine Drugs*, 13 (6): 3422-3442.

Suganya, S., R. Ishwarya, R. Jayakumar, M. Govindarajan, N.S. Alharbi, S. Kadaikunnan, J.M. Khaled, M.N. Al-Anbr and B. Vaseeharan (2019). New insecticides and antimicrobials derived from *Sargassum wightii* and *Halimeda gracillis* seaweeds: Toxicity against mosquito vectors and antibiofilm activity against microbial pathogens. *South African Journal of Botany*, 125: 466-480.

Tariq, R.M. and S.S. Qadri (2001). Repellant activity of some local plant's oil, two commercial repellants, di-methyl phthalate and non-alcoholic itter against dengue vector mosquitoes. *Pakistan Journal of Entomology*, 16: 7-10.

Usmanghani, K., M. Shameel, S. Siddiqui and M. Alam (1987). Studies on the sterols of a brown seaweed *Iyengaria stellata* from Pakistan. *Pakistan Journal of Botany*, 19 (2): 249-252.

Vimaladevi, S., A. Mahesh, B.N. Dhayanithi and N. Karthikeyan (2012). Mosquito larvicidal efficacy of phenolic acids of seaweed *Chaetomorpha antennina* (Bory) Kuetz. against *Aedes aegypti*. *Biologia*, 67: 212-216.

Yogarajalakshmi, P., T.V. Poonguzhali, R. Ganesan, S. Karthi, S. Senthil-Nathan, P. Krutmuang, N. Radhakrishnan, F. Mohammad, T.J. Kim and P. Vasantha-Srinivasan (2020). Toxicological screening of marine red algae *Champia parvula* (C. Agardh) against the dengue mosquito vector *Aedes aegypti* (Linn.) and its non-toxicity against three beneficial aquatic predators. *Aquatic Toxicology*, 222: 105474.

Yu, K.X., C.L. Wong, R. Ahmad and I. Jantan (2015). Mosquitocidal and oviposition repellent activities of the extracts of seaweed *Bryopsis pennata* on *Aedes aegypti* and *Aedes albopictus*. *Molecules*, 20 (8): 14082-14102.

(*Accepted for publication March 2024*)