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Studies on Ethanolic Extract of Brown Seaweed *Turbinaria ornata* from Coastal Area of Tamil Nadu: *In vitro* Antioxidant and Anticancer Properties

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Seaweeds are one of the most diverse bioreserves, including a wide range of bioactive compounds with various properties and functions. In present study, the antioxidant and anticancer activity of brown seaweed *Turbinaria ornata* was extracted using two different solvents like aqueous and ethanol. Higher concentrations of bioactive compounds were observed in ethanolic extract of *T. ornata* (EETO) in comparison to aqueous extract of *T. ornata* (AETO). The ethanolic extract showed potent antioxidant properties for DPPH and hydroxyl radical scavenging. The IC_{50} value of the ethanolic extract was found to be 48 and 50 µg/mL for DPPH and hydroxyl radical scavenging assay, respectively. Human breast adenocarcinoma (MCF-7) cells treated with different concentrations of the ethanolic extract showed a dose-dependent increase in cell inhibition. The LC_{50} value of the ethanolic extract was calculated to be 115.19 µg/mL and cell morphology was observed under an inverted phase-contrast microscope. As a result, based on the present findings, it is suggested that *T. ornata* extract might be a promising antioxidant and anticancer therapeutics.

Keywords: *Turbinaria ornata,* Flavonoids, Human breast adenocarcinoma cells, Anticancer activity.

INTRODUCTION

Brown seaweed is known as macroalgae is acquiring major relevance due to the synthesis of novel bioactive compounds such as fatty acids, polysaccharides, alkaloids, terpenes, steroids, heterocyclic carbons and cyclic peptides. The majority of these metabolites have some physiological actions such as antioxidant, anti-inflammatory, antimalarial, antimicrobial, antiviral and anticancer [1,2]. Cancer is a group of disorders in which cells continue to develop uncontrollably, spread into adjacent tissues and form tumors. A poor diet, drug use, infectious organisms, environmental pollutants, inherited genetic mutations, hormones and immunological disorders are all variables that might cause cancer [3] and is regarded as one of the world's leading causes of death [4]. In 2018, an estimated 18.1 million new instances of cancer were identified, with 9.6 million cancer deaths, making cancer one of the leading causes of morbidity and mortality in humans. The most common forms of cancer are breast, lung and colorectal cancers, which account for the majority of cancer cases worldwide [5-8].

Until now, there has been no safe cancer treatment because the currently available medications cause side effects such as vomiting, nausea, diarrhea and exhaustion. As a result, it's critical to investigate and find new anticancer drugs derived from natural sources that are safe, inexpensive and less harmful [1]. Marine sources are the most common and produce high-quality bioactive metabolites. A total of 20,000 compounds have been reported from algae, as well as 12,000 compounds from other tiny members of marine plants, animals and other sources, excluding bacteria [9]. Majority of pharmaceuticals are made from microbes, herbal plants and seaweeds. However, macroalgae can be thought of as natural bioactive chemical factories.

Brown seaweeds have more antioxidant capabilities than red and green seaweeds [10]. *Turbinaria ornata*, brown seaweed, is the most important in biomedical and biopharmaceutical research [11]. It contains antioxidants, antimicrobial, anti-fungal, larvicidal and anticancer properties [12]. Based on the foregoing, the current work analyzing the phytochemical compounds in two solvents *e.g.* aqueous and ethanol. Further, an

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ethanolic extract of *T. ornata* (EETO) to conduct quantitative phytochemical screening and to identify the active phytochemical components responsible for antioxidant activity. *In vitro* cytotoxicity and cell morphology experiments against human breast adenocarcinoma (MCF-7) cells were also undertaken to determine their anticancer properties.

EXPERIMENTAL

Cell lines: MCF-7 (human breast adenocarcinoma) cells were initially purchased from National Centre for Cell Science (NCCS), Pune, India. Dulbecco's Modified Eagle's Medium (DMEM) medium (Sigma-Aldrich, USA), fetal bovine serum (FBS) and antibiotic solution were purchased from Merck, Germany. 1,10-Phenanthroline and 2,2-diphenyl-1-picryl hydrazyl (DPPH) were obtained from Sigma Chemical Co., USA. The rest of the chemicals and solvents were of Analytical grade.

Brown algae: Fresh brown seaweeds of *T. ornata* were collected from the coastal area of Rameswaram town, India. *T. ornata* (Ref No.: BSI/SRC/5/23/2015/Tech/920) algae sample was identified and authenticated by Dr. M. Palanisamy, Scientist D, Botanical Survey of India, Southern region centre, Tamil Nadu Agriculture University Campus, Coimbatore, India. The *T. ornata* was carefully rinsed with fresh water and dehydrated in the shade at room temperature for a week. The dehydrated *T. ornata* sample was powdered and stored at –20 ºC.

Preparation of *T. ornata* extracts: The *T. ornata* extract was prepared using two different solvents (aqueous and ethanol). Powdered *T. ornata* sample (10 g) in two conical flasks along with added 500 mL of distilled water and absolute ethanol, respectively. Both extracts were shaken well for 0.5 h by rotary shaker and kept for 24 h. After 24 h, extractions were filtered using Whatman No.1 filter paper and the filtrate was further used for analysis.

Phytochemical screening: Phytochemical screening of both aqueous and ethanol extract of *T. ornata* was carried out qualitatively for the presence of polyphenols, flavonoids, cardiac glycosides, alkaloids, anthraquinones, terpenes, tannins, saponins, coumarins, emodins, anthocyanins and steroids according to the reported method [13-15]. Further, the total phenols and total flavonoids content in the ethanolic extract were quantitatively determined according to the reported method [16,17] and the values were expressed as mg/g.

***In vitro* reactive oxidants scavenging activities:** The reactive oxidants scavenging activity was carried out by a reported method with slight modifications [18,19]. The DPPH radical scavenging activity was carried out using different concentrations of (20-80 µg/mL) ethanolic extract of *T. ornata* (EETO) or ascorbic acid (standard). The reaction mixture was incubated with 100 µM of DPPH in 2 mL of methanol for 30 min in dark conditions. The DPPH devoid of test samples served as control. The decrease in absorbance was read at 517 nm using a UV-vis spectrophotometer (UV-1800, Shimadzu, Torrance, USA). For hydroxyl radical scavenging assay, various concentrations of (20-80 µg/mL) EETO or ascorbic acid (standard) was added to the reaction mixture [$FeCl_3$ (1.0 mM), 1,10-phenanthroline (1 mM), 0.2 M phosphate buffer (pH 7.8), H_2O_2 (0.17 M)] and incubated for 5 min at room temperature. The absorbance was measured at 560 nm using a UV-vis spectrophotometer.

Determination of cell viability by MTT assay: MCF-7 cells were cultured in liquid medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin B and maintained under 5% CO_2 incubator at 37 ºC. All the assays were carried out at 80% confluence of the cells. For cytotoxicity analysis, MCF-7 cells were treated with various concentrations (25-100 µg/mL) of EETO for 24 h. After the treatment, the liquid portion of the media was removed, then 30 µL of MTT solution was added to each well and incubated for 4 h at 37 ºC in a CO_2 incubator. Later, the medium containing MTT was removed from each well and replaced with 100 µL of DMSO to dissolve the dark blue formazan formed in surviving cells. Then, the absorbance was read using a microplate reader at a wavelength of 540 nm. After MTT treatment, the structural changes around the surface in MCF-7 cells were examined. In the present study, cellular changes in MCF-7 were initiated by EETO. All kinds of cells were exposed to various concentrations (25-200 µg/mL) of EETO. Finally, the cells from treated groups were subjected to wash and observed under an inverted phase-contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera). Each experiment was carried out twice and in triplicate [20,21].

RESULTS AND DISCUSSION

Preliminary phytochemical screening: The qualitative phytochemical screening results showed that saponins, steroids, polyphenols, glycosides, tannins, alkaloids, anthraquinones, coumarins, emodins and anthocyanins were present in both extracts *i.e*. AETO and EETO (Table-1). In comparison, EETO showed a higher concentration of phytochemicals particularly, flavonoids and terpenoids than AETO. Due to the significant concentration of phytochemicals in EETO, it is subjected to further screening. The predominant concentration of polyphenols and flavonoids in EETO was proved by quantitative phytochemical screening. The total phenolic content (TPC) and total flavonoid content (TFC) in EETO were found to be 179.52 ± 12.56 mg/g and 122.23 ± 8.54 mg/g, respectively. Sivasankaran *et al*. [22] stated that the highest concentration of TPC and TFC in *T. ornata* is could be used as an antibiotic. These phytochemicals initiate their potential as bioactive compounds. Wu *et al*. [23] reported that the phenolic compounds are one of the most effective antioxidants in brown algae. Muhammad *et al*. [24] exhibited that at the highest concentration (3 mg/mL), an ethanolic extract of *Sargassum polycystum* had the highest total phenolic content (627 ± 50.81 mg GAE/100 g dry samples). These findings suggested that phenolic chemicals played a significant role in antioxidant activity.

***In vitro* reactive oxidants scavenging activities:** In the DPPH scavenging assay, EETO showed 85% of DPPH radical scavenging activity at 80 µg/mL concentration. The IC_{50} value of EETO for DPPH scavenging activity was 48 µg/mL. The IC_{50} value of ascorbic acid (standard) for DPPH scavenging activity was 44 µg/mL. The EETO showed potent antioxidant

TABLE-1
PHYTOCHEMICAL SCREENING OF EETO

Phytochemicals	EETO	AETO
Tannin	Moderate presence	Moderate presence
Saponins	Higher presence	Higher presence
Flavonoids	Higher presence	Moderate presence
Steroids	Higher presence	Higher presence
Terpenoids	Higher presence	Moderate presence
Triterpenoids	Higher presence	Moderate presence
Alkaloids	Absence	Absence
Antroquinone	Moderate presence	Moderate presence
Polyphenol	Higher presence	Higher presence
Glycosides	Higher presence	Higher presence
Coumarins	Moderate presence	Moderate presence
Emodins	Absence	Absence
Anthocyanins	Absence	Absence

activity which was comparable to that of standard ascorbic acid as shown in Fig. 1a. The percentage scavenging effect of EETO was found to be increased dose dependently. Nazarudin *et al*. [24] reported that an ethanolic extract of *Sargassum polycystum* had the highest DPPH scavenging activity (61.4 ± 0.171%). Alharbi *et al*. [25] exhibited that the EETO showed excellent total antioxidant activity and DPPH scavenging activity at higher concentration of 250 µg/mL. The Moroccan seaweed *Fucus spiralis* showed significant DPPH scavenging activity with IC_{50} value of 47.23 ± 3.8 µg/mL [26]. Aminina *et al*. [27] revealed that the free radical scavenging effect of the bioactive compound phlorotannins is one of the polyphenols, determined by phenolic hydroxyl groups attached to a ring structure, which act as electron traps are reliable with higher scavenging effect among highest polyphenolic concentration in brown algae.

The hydroxyl radical ($OH^{\bullet}$) is the most reactive and toxic ROS ever discovered. The Fenton reaction between H_2O_2 and O_2 is catalyzed by transition metals like Fe (Fe^{2+}, Fe^{3+}) at neutral pH. It has the ability to cause lipid peroxidation (LPO), protein degradation and membrane breakdown in several cellular components. Excess aggregation of $OH^{\bullet}$ causes cellular death because there is no enzymatic machinery to scavenge this harmful radical [28]. As well, in hydroxyl radical scavenging, the percentage scavenging of EETO was increased in a dose-dependent manner (Fig. 1b). The EETO showed 82% of hydroxyl radical scavenging activity at 80 µg/mL concentration. The IC_{50} value of EETO for hydroxyl radical scavenging activity was 50 µg/mL. The hydroxyl radical quenching effect of EETO was comparable to that of ascorbic acid (standard). The most reactive hydroxyl radical has a short half-life, can remove hydrogen atoms by radicals from cell membranes and cause oxidative degradation of lipids [29]. In present study, EETO showed a significant effect on DPPH and hydroxyl radical scavenging it might be due to the occurrence of bioactive compounds like flavonoid and tannins synergistically contribute to the antioxidant property of EETO.

Anticancer activity: MCF-7 cell line was used to study the anticancer effect of different concentrations (25-200 µg/mL) of EETO by MTT reduction assay and 5-fluorouracil was used as a positive control. MCF-7 cells treated with different concentrations of EETO showed increasing cell inhibition in a dose-dependent manner. The result showed that the anticancer effect of EETO was 76% (Fig. 2). Decreased percentage of cell viability has been observed in increasing concentration of EETO, as proved by cell death. The LC_{50} (lethal concentration 50 %) value of EETO in MCF-7 was found to be 115.19 µg/mL (Table-2). In this study, the EETO showed significant anticancer activity toward the MCF-7 cells.

The morphological changes in MCF-7 cells were observed by an inverted phase-contrast microscope (Fig. 3). In control, an opaque cell layer was observed. Furthermore, MCF-7 cells treated with the EETO were noticed with low density and fragmented cells owing to apoptotic cell death. The development of apoptotic cells could be caused by changes in nuclear content as well as mitochondrial membrane potential. Because of their ability to prevent mitotic cell division, terpenoids are regarded interesting bioactive chemicals in the quest for anticancer medicines [30]. Many studies have been reported on the anticancer activity of brown seaweed *Turbinaria* sp. utilizing different extracts on various cancer cells. *Turbinaria* sp. does, in fact,

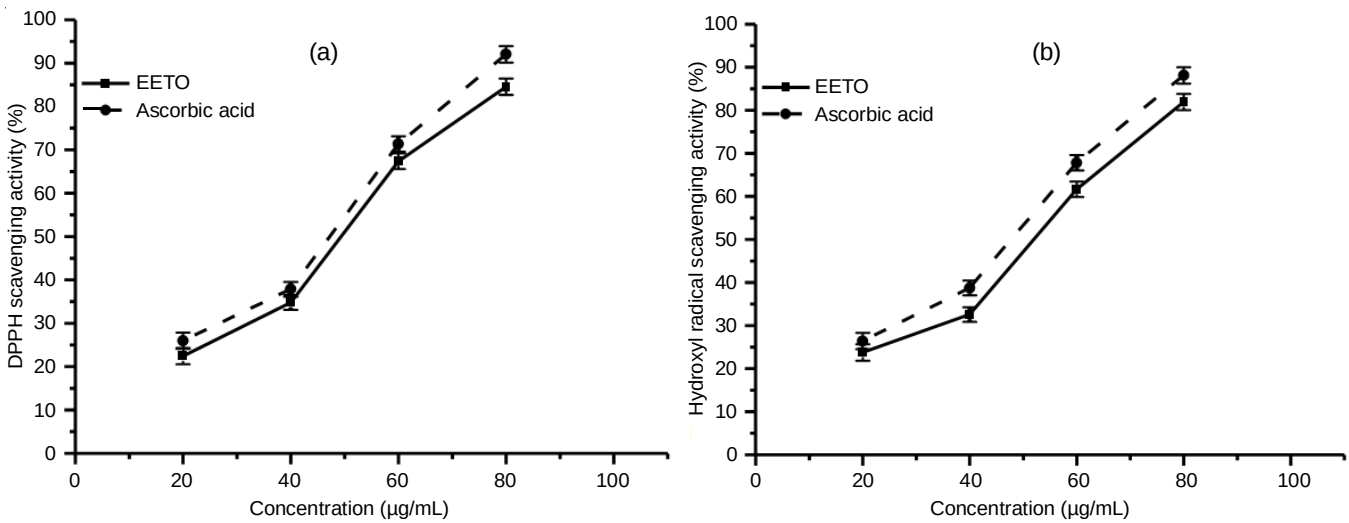

Fig. 1. Antioxidant scavenging effect of EETO. DPPH radical scavenging activity (a) and hydroxyl radical scavenging activity (b) of EETO are shown. Results are expressed as mean ± SD (n = 6)

TABLE-2
EFFECT OF DIFFERENT CONCENTRATIONS OF EETO ON VIABILITY AGAINST MCF-7 CELL

Activity	Cell control	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)	200 (µg/mL)
Viability (%)	100	85.42	70.56	40.12	24.45
LC_{50} value (µg/mL)			115.19		

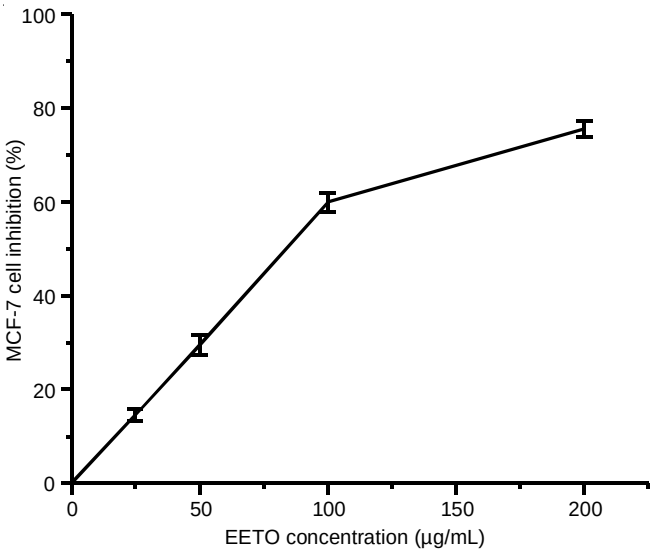

Fig. 2. Anticancer activity of EETO against MCF-7 cells

have varying dosage effects depending on the formulation. Ethyl acetate fraction of *T. conoides* showed significant anticancer activity against HepG2 cell line, cell growth inhibition was found to be 67% at 24 h and 83% at 48 h [31]. Ethanolic extract of *Padina australis* and *T. ornata* exhibited significant cytotoxicity against baker's yeast on highest concentration with an IC_{50} value was found to be 0.54 mg/mL and 0.53 mg/mL, respectively [32]. Bharath *et al*. [33] reported that *in vitro* anticancer activity of hexadecanoic acid of *T. ornata* has a significant cell growth inhibitory action against HT-29 human colon cancer cells, with an IC_{50} value of 36.04 µg/mL.

According to Mofeed *et al*. [1], *Ulva lactuca* (88.5 ± 1.08%) and *Amphiroa anceps* (86.1 ± 2.88%) extracts had the highest inhibitory percentages against MCF-7 and colorectal carcinoma cell lines (HCT-116) in *in vitro* anticancer activity. Brown seaweed extracts were found to be cytotoxic to the MCF-7 cell line with IC_{50} values ranging from 3.54 ± 1.2 to 21.2 ± 1.1 µg/mL. Overall, this study demonstrates that due to the existence of bioactive compounds, the biological functions of EETO provide a remarkable approach for the treatment of numerous disorders.

Conclusion

The curative effect of brown seaweed *Turbinaria ornata* was investigated due to the presence of bioactive compounds. The present study findings represented that an ethanolic extract of *T. ornata* (EETO) showed the promising anticancer activity against MCF-7 cells at higher concentrations. In addition, EETO contains bioactive compounds like flavonoids and terpenes may contribute to antioxidant and anticancer activity. Thus, the purification of bioactive compounds from *T. ornata* sp. and its mechanism by which the compound induce an antiproliferation effect required to be considered in advance studies to assist the strong application of these novel anticancer agents.

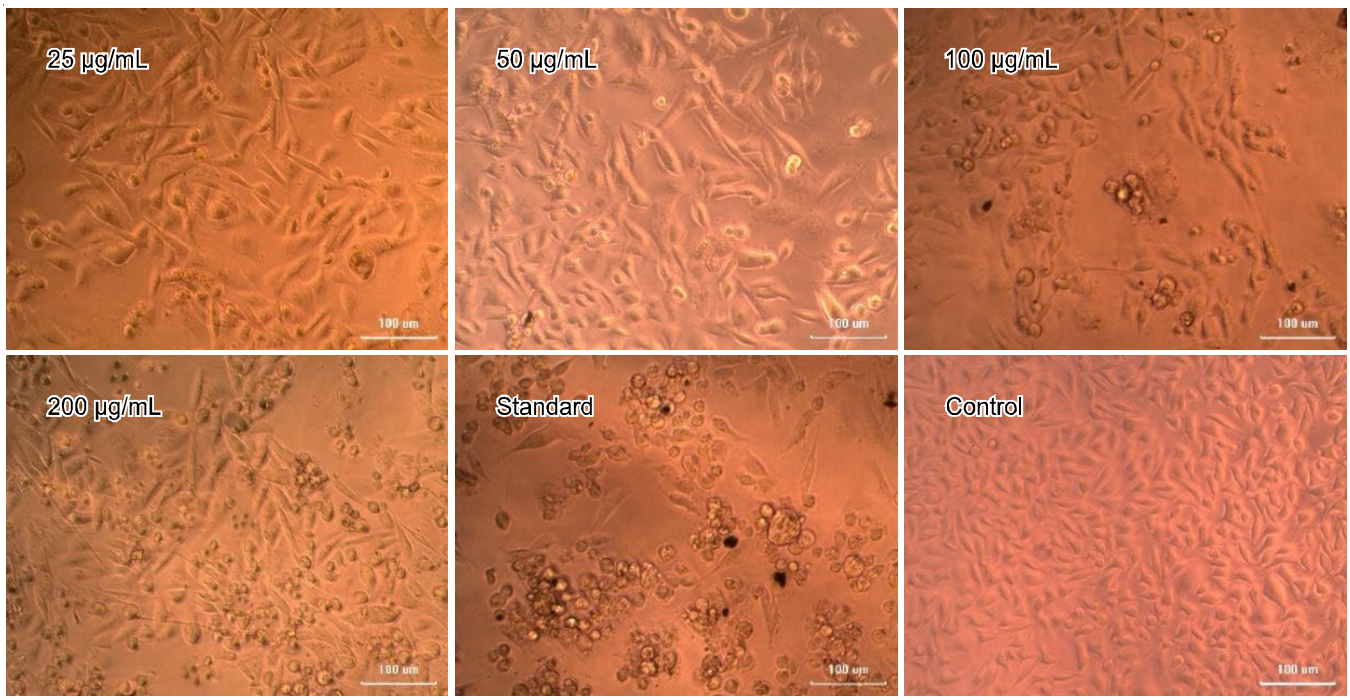

Fig. 3. Morphological changes in MCF-7 cells by the exposure of different concentrations of EETO for 24 h. Images were recorded under inverted phase contrast microscope. *$p < 0.05$ *versus* control

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. J. Mofeed, A. Sabry and M.A. Deyab, *Biosci. Res.*, **16**, 3801 (2019).
2. M. Deyab, J. Mofeed, E. El-Bilawy and F. Ward, *Arch. Microbiol.*, **202**, 213 (2020); https://doi.org/10.1007/s00203-019-01734-9
3. American Cancer Society, What Is Cancer? American Cancer Society (2017).
4. I. Sheikh, V. Sharma, H.S. Tuli, D. Aggarwal, A. Sankhyan, P. Vyas, A.K. Sharma and A. Bishayee, *Biointerface Res. Appl. Chem.*, **11**, 8502 (2020); https://doi.org/10.33263/BRIAC111.85028537
5. F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre and A. Jemal, *CA Cancer J. Clin.*, **68**, 394 (2018); https://doi.org/10.3322/caac.21492
6. E.M. Brown, P.J. Allsopp, P.J. Magee, C.I.R. Gill, S. Nitecki, C.R. Strain and E.M. McSorley, *Nutr. Rev.*, **72**, 205 (2014); https://doi.org/10.1111/nure.12091
7. J. Cotas, V. Marques, M.B. Afonso, C.M.P. Rodrigues and L. Pereira, *Mar. Drugs*, **18**, 50 (2020); https://doi.org/10.3390/md18010050
8. D.M. Parkin, F. Bray, J. Ferlay and P. Pisani, *CA Cancer J. Clin.*, **55**, 74 (2005); https://doi.org/10.3322/canjclin.55.2.74
9. J. Mofeed, *Egypt. Acad. J. Biol. Sci.*, **11**, 89 (2019); https://doi.org/10.21608/eajbsg.2019.85014
10. L.X. Zheng, X.Q. Chen and K.L. Cheong, *Int. J. Biol. Macromol.*, **151**, 344 (2020); https://doi.org/10.1016/j.ijbiomac.2020.02.168
11. M.M. El-Sheekh, R.A.E.K. El-Shenody, E.A. Bases and S.M. El-Shafay, *Food Sci. Technol.*, **41(suppl 1)**, 29 (2021); https://doi.org/10.1590/fst.06120
12. C.J. Agrawal, S.K. Barrow and S.K. Deshmukh, *Microb. Pathog.*, **146**, 104248 (2020); https://doi.org/10.1016/j.micpath.2020.104248
13. A. Sofowara, Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd.: Ibadan, Nigeria. p. 289 (1993).
14. G.E. Trease and W.C. Evans, Pharmacognsy, Ed.: 11, Brailliar Tiridel Canada: Macmillian Publishers (1989).
15. J.B. Harborne, Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis, Ed.: 2, London: Chapman and Hall Ltd., pp. 49-188 (1973).
16. H.O. Edeoga, D.E. Okwu and B.O. Mbaebie, *Afr. J. Biotechnol.*, **4**, 685 (2005); https://doi.org/10.5897/AJB2005.000-3127
17. B.A. Boham and R. Kocipai-Abyazan, *Pacific Sci.*, **48**, 458 (1974).
18. K. Shimada, K. Fujikawa, K. Yahara and T. Nakamura, *J. Agric. Food Chem.*, **40**, 945 (1992); https://doi.org/10.1021/jf00018a005
19. F. Yu, F. Yu, R. Li and R. Wang, *J. Ethnopharmacol.*, **95**, 77 (2004); https://doi.org/10.1016/j.jep.2004.06.025
20. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983); https://doi.org/10.1016/0022-1759(83)90303-4
21. A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo and M. Boyd, *J. Natl. Cancer Inst.*, **83**, 757 (1991); https://doi.org/10.1093/jnci/83.11.757
22. S. Ramarajan, V. Janakiraman and Allan, *Res. J. Pharm. Technol.*, **12**, 108 (2019); https://doi.org/10.5958/0974-360X.2019.00021.0
23. Q. Wu, L. Liu, A. Miron, B. Klimova, D. Wan and K. Kuca, *Arch. Toxicol.*, **90**, 1817 (2016); https://doi.org/10.1007/s00204-016-1744-5
24. M.F. Nazarudin, A. Paramisparam, N.A. Khalid, M.N. Albaz, M.S. Shahidan, I.S. Md Yasin, A. Isha, M. Abu Zarin and M. Aliyu-Paiko, *Arabian J. Chem.*, **13**, 7652 (2020); https://doi.org/10.1016/j.arabjc.2020.09.002
25. N.S. Alharbi, S.A. Alyahya, G. Ramachandran, C.K. Chelliah, S. Kadaikunnan, J.M. Khaled, K.F. Alanzi, G. Rajivgandhi and N. Manoharan, *J. King Saud Univ. Sci.*, **32**, 3447 (2020); https://doi.org/10.1016/j.jksus.2020.10.005
26. F. Grina, Z. Ullah, E. Kaplaner, A. Moujahid, R. Eddoha, B. Nasser, P. Terzioglu, M.A. Yilmaz, A. Ertas, M. Öztürk and A. Essamadi, *S. Afr. J. Bot.*, **128**, 152 (2020); https://doi.org/10.1016/j.sajb.2019.10.021
27. N.M. Aminina, E.P. Karaulova, T.I. Vishnevskaya, E.V. Yakush, Y.-K. Kim, K.-H. Nam and K.-T. Son, *Molecules*, **25**, 3909 (2020); https://doi.org/10.3390/molecules25173909
28. E. Pinto, T. Sigaud-kutner, M.A. Leitao, O.K. Okamoto, D. Morse and P. Colepicolo, *J. Phycol.*, **39**, 1008 (2003); https://doi.org/10.1111/j.0022-3646.2003.02-193.x
29. S. Vladimir-Kne•evic, B. Bla•ekovic, M. Bival Štefan, A. Alegro, T. Kõszegi and J. Petrik, *Molecules*, **16**, 1454 (2011); https://doi.org/10.3390/molecules16021454
30. A.J. Smit, *J. Appl. Phycol.*, **16**, 245 (2004); https://doi.org/10.1023/B:JAPH.0000047783.36600.ef
31. A. Ponnan, K. Ramu, M. Marudhamuthu, R. Marimuthu, K. Siva and M. Kadarkarai, *Clin. Phytosci.*, **3**, 5 (2017); https://doi.org/10.1186/s40816-017-0042-y
32. J.L. Canoy and J.G. Bitacura, *Anal. Cellul. Pathol.*, **2018**, 3709491 (2018); https://doi.org/10.1155/2018/3709491
33. B. Bharath, K. Perinbam, S. Devanesan, M.S. AlSalhi and M. Saravanan, *J. Mol. Struct.*, **1235**, 130229 (2021); https://doi.org/10.1016/j.molstruc.2021.130229