http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2023.403.408

Research Article
In vivo Anti-Inflammatory and Immunomodulatory Activity of Soft Coral *Nephthea* sp. from Southeast Sulawesi

[1]Idin Sahidin, [5]Baru Sadarun, [2]Wahyuni Wahyuni, [3]La Ode Muhammad Julian Purnama, [4]Nur Syifa Rahmatika, [1,3]Muhammad Hajrul Malaka, [2]Fadhliyah Malik and [2]Adryan Fristiohady

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Halu Oleo University, Kendari 93232, Indonesia
[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Halu Oleo University, Kendari 93232, Indonesia
[3]Thammasat University Research Unit in Drug, Health Product Development and Application, Faculty of Pharmacy, Thammasat University, Bangkok 10200, Thailand
[4]Faculty of Medicine, Halu Oleo University, Kendari 93232, Indonesia
[5]Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232, Indonesia

Abstract

Background and Objective: *Nephthea* sp., has various biological activities. The study on anti-inflammatory and immunomodulatory of *Nephthea* sp., from Southeast Sulawesi is still limited. Hence, this study aims to determine the content of secondary metabolite compounds and their pharmacological activities including anti-inflammatory and immunomodulatory. **Materials and Methods:** *Nephthea* sp., was collected from Saponda Island and extracted using ethyl acetate. The chemical contents were analyzed by a phytochemical screening test, anti-inflammatory activity by xylene-induced ear edema and immunomodulatory activity using macrophage phagocytic activity (SPA) in experimental animals. **Results:** The ethyl acetate extract of *Nephthea* sp., contains flavonoids and steroids. According to the result obtained, the ethyl acetate extract of *Nephthea* sp., exhibited anti-inflammatory and immunomodulatory activity *in vivo*. The EAN 0.2 demonstrated the highest potency and showed no significant difference compared to diclofenac sodium at a concentration of 0.15 mg mL^{-1} ($p>0.05$) with the highest percentage edema inhibition as in xylene-induced ear edema. In addition, EAN 0.2 exhibited a similar result in increasing SPA compared to control ($p>0.05$). Both assays showed significant differences with negative control in this study ($p<0.05$). **Conclusion:** Soft coral *Nephthea* sp., can be a potential candidate as an anti-inflammatory and immunomodulatory agent.

Key words: *Nephthea* sp., Southeast Sulawesi, ethyl acetate extract, anti-inflammatory, immunomodulatory

Citation: Sahidin, I., B. Sadarun, W. Wahyuni, L.O.M.J. Purnama and N.S. Rahmatika *et al*., 2023. *In vivo* anti-inflammatory and immunomodulatory activity of soft coral *Nephthea* sp. from Southeast Sulawesi. Pak. J. Biol. Sci., 26: 403-408.

Corresponding Author: Idin Sahidin, Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Halu Oleo, Kendari 93232, Indonesia
Adryan Fristiohady, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Halu Oleo, Kendari 93232, Indonesia Tel: (+62) 811 410 1234

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia is the largest archipelagic country in the world, with 17,500 islands and a coastline of 81,000 km. In addition, the water area in Eastern Indonesia has high marine biodiversity[1,2]. Sulawesi is located in the eastern area of Indonesia with abundant biodiversity, including soft coral, which has the potential for bioactive substances and biological activity[3].

Soft corals are marine biotas rich in biologically active substances[4]. *Nephthea* sp. (Nephtheidae) is one of the soft corals that are widely distributed in the Indo-Pacific Region[5]. Soft corals belonging to the Nephtheidae family have several chemical compounds with antibacterial, antiviral, antidiabetic, anti-inflammatory and anticancer activities[6]. Several compounds have been identified, including sesquiterpenes, diterpenes, sterile and quinones[7].

Previous studies showed that the methanol extract of *Nephthea* sp., has activity as an anticancer due to the presence of diterpene compound in it[8]. In addition, nephteoxydiol from *Nephthea* sp., inhibits the growth of melanoma cancer cells[9]. *Nephthea* sp., also has nephalsterol A, which has cytotoxic properties against tumor cell proliferation[10]. *Nephthea* sp., also has anti-inflammatory activity due to the presence of steroids and terpenoids, including nebrosteroids A-H and columnariols A and B[7,11,12]. Similarly, Nebrosteroid I and Chabrosterol have activity as anti-inflammatory and immunomodulators by inhibiting the expression of iNOS and COX-2 in macrophages[13].

The studies exploring *Nephthea* sp., as anti-inflammatory and immunomodulatory are still limited, especially *Nephthea* sp., originating from Southeast Sulawesi. Therefore, it is necessary to study the potency of *Nephthea* sp., as a novel candidate for anti-inflammatory and immunomodulatory agents.

MATERIALS AND METHODS

Study area: The experiment was conducted from January, 2023 to June, 2023 at the Faculty of Pharmacy, Halu Oleo University, Indonesia.

Sample collection: The samples used were soft coral *Nephthea* sp. It was collected from Saponda Islands in Southeast Sulawesi, Indonesia at a slope reef depth of 4-10 m. Samples collected (1.1 kg) were put in an ice container and later kept at -20°C for further analysis.

Extraction: *Nephthea* sp. (1.1 kg) samples were chopped into small pieces and extracted in 10 L of ethyl acetate (3×24 hrs) at room temperature. Then, the concentrated extract obtained was 36.8 g (3.2%).

Phytochemical screening: The phytochemical screening of *Nephthea* sp. (EAN) was performed qualitatively by modified Harborne colorimetric methods. It aims to identify the presence of alkaloids, flavonoids, steroids/terpenoids, tannins and saponins:

- **Alkaloid:** About 1 g of EAN was dissolved with ethanol in a tube and mixed thoroughly with the Dragendorff reagent. Brown sediment formed indicated the presence of alkaloids
- **Flavonoid:** About 1 g of EAN was dissolved with ethanol. Thereafter, the EAN solution was mixed with 0.2 mg of magnesium powder and 1 mL of HCl. The color change into red, orange, yellow and green indicated the presence of flavonoids
- **Steroid/terpenoid:** About 1 g of EAN was dissolved in ethanol and mixed with CH_3COOH (1 mL) and H_2SO_4 (1 mL). The presence of steroid was indicated by the immiscible reddish brown ring, while the terpenoid was an immiscible blue or greenish ring
- **Tannin:** About 1 g of EAN was dissolved with ethanol and added with 5% $FeCl_3$ (3 drops). The color change into blackish green or navy indicated the presence of tannin
- **Saponin:** About 1 g of EAN was dissolved in 10 mL of distilled water in a tube test. Thereafter, the tube was vigorously shaken with vortex until the foam. The presence of saponin was indicated by stable foam in the tube

Acclimatization of experimental animals: The experimental animals used were mice obtained from an animal farm in Surabaya, East Java, Indonesia. The animals were acclimatized for 1 week prior experiment at 25±1°C, Rh 55±5% and 12:12 hrs light/dark cycle. The animals were also allowed to access the food and water *ad libitum*.

Anti-inflammatory activity: Experimental animals (n = 24) were induced with topical xylene by applying it to the dorsal surface of the right ear of mice at a dose of 30 µL. Edema degree was calculated by the difference between the thickness of the right ear (with edema) and the left ear (without edema)[14]. After that, the animals (n = 24) were divided into six groups to receive treatment as follows:

- **Group I:** Normal group, which did not receive any treatment
- **Group II:** Negative control, which received 0.5% Na-CMC suspension
- **Group III:** Positive control, which received diclofenac sodium (0.15 mg mL^{-1})

- **Group IV:** EAN 0.05 mg mL^{-1}
- **Group V:** EAN 0.1 mg mL^{-1}
- **Group VI:** EAN 0.2 mg mL^{-1}

The observation was conducted at 0, 15, 30 and 45 min. The edema percentage (Edema %) was calculated as follows:

$$\text{Edema (\%)} = \frac{T_t - T_0}{T_0} \times 100$$

where, T_t represents ear thickness at t minutes and T_0 represents ear thickness at 0 min. From % edema, the percentage of inflammatory inhibition (% inflammatory inhibition) was calculated by following the formula[15]:

$$\text{Inflammatory inhibition (\%)} = \frac{\text{Edema control} - \text{Edema treated}}{\text{Edema control}} \times 100$$

Immunomodulator activity: In this experiment, mice were used as experimental animals. After acclimatization, the experimental animals used (n = 30) were divided into 4 groups, which were groups I, II, III and IV). Then, they were treated orally for 7 days consecutively with the following treatment:

- **Group I:** Positive control (Stimuno®1000)
- **Group II:** EAN 0.05
- **Group III:** EAN 0.1
- **Group IV:** EAN 0.2

After 7 days, the animals were infected by *Staphylococcus aureus* intraperitoneally for 1 hr. Then, animals were sacrificed and the peritoneal solution was collected. It was followed by observing in object glass after fixating with methanol (Merck®) and colored by 10% Giemsa (Merck®). The immunomodulatory activity was calculated as the specific phagocytic activity (SPA) of macrophages originating from peritoneal solution under the light microscope (Olympus®) with magnification from 10-1000X. The SPA was calculated by following as follow[16]:

$$\text{SPA (\%)} = \text{(Active macrophages:Total macrophages)x100}$$

Statistical analysis: Data collected is presented as a mean±SD. Statistical analysis was performed at a significance level of /0.05 by using a One-way Analysis of Variance (ANOVA) with Tukey's multiple comparisons.

RESULTS

Phytochemical screening: The results of the chemical content test showed that the ethyl acetate extract of *Nephthea* sp., positively contains flavonoids, alkaloids, tannins, saponins and terpenoids.

Effect of *Nephthea* sp., on xylene-induced mice ear edema: Xylene induction was performed on mice's ears topically. Shortly after 15 min of induction, mice immediately showed signs of inflammation, one of which was swelling (tumor). Saputri and Zahara[17], studied that swelling in the inflammatory response is caused by fluid accumulation in mice's ears. After 15, 30 and 45 min, the thickness of the edema in the ears of mice was measured. The average measurement with a thickness gauge meter can be seen in Fig. 1.

The thickness of the edema indicates the inflammation process. Figure 2 shows that the average thickness of edema in the ears of mice starts from the largest, namely the control group of 2.85 mm, the diclofenac group, EAN 0.05 and EAN 0.1 of 2.82 mm and the EAN group 0.2 by 2.81 mm.

At 30 and 45 min after treatment, each group was measured again for the final ear thickness of the mice to know the effect of carriers, drug administration and extracts on reducing edema. The thickness of the edema at 45 min showed no decrease in the thickness of the edema for the control group. It indicates that the control group did not have an anti-inflammatory effect. The diclofenac group showed the lowest edema thickness of 0.97 mm, meaning that the positive control group was the most effective in reducing inflammation compared to other groups. It was followed by EAN 0.1 of 1.26 mm, EAN 0.2 of 1.29 mm and EAN 0.05 of 1.75 mm.

The anti-inflammatory effect in the percentage of inflammatory inhibition (%) was shown in Fig. 2. The highest percentage of inflammation inhibition was diclofenac group, EAN 0.05, EAN 0.1 and EAN 0.2, by 56.48, 28.51, 39.43 and 47.15%, respectively. Figure 2 showed that each sample group differed significantly from the diclofenac group ($p<0.05$ and $p = 0.000$). The EAN 0.2 provided the highest inflammatory inhibition (%), thus concluding that EAN 0.2 had better anti-inflammatory activity than EAN 0.05 and EAN 0.1, yet less than the diclofenac group.

Immunomodulatory activity: An immunomodulatory activity assay was conducted by measuring the phagocytic activity. As seen in Fig. 3, it found that the average percentage of phagocytic activity of macrophages was negative control,

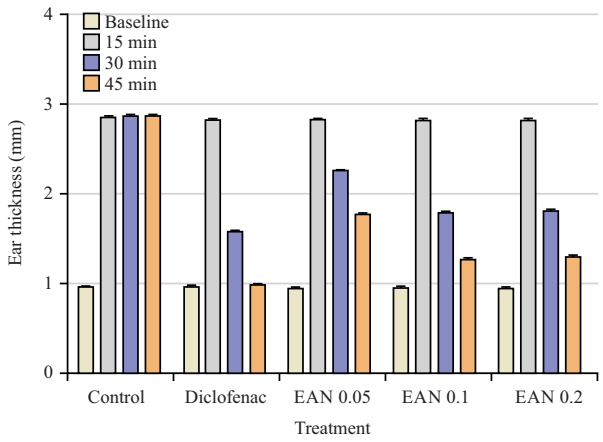

Fig. 1: Thickness of mice ear post induced with xylene against time
Data is presented as mean±time

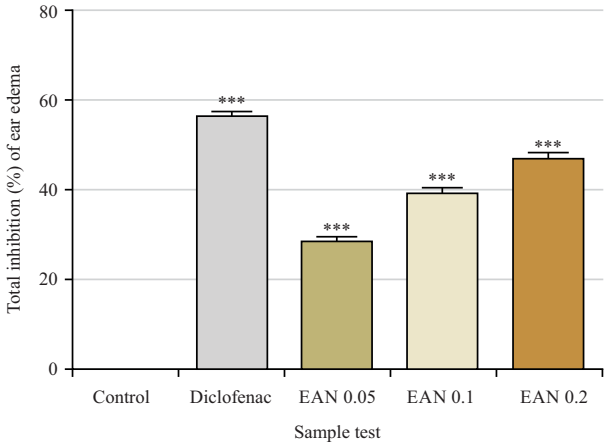

Fig. 2: Total inhibition (%) of ear edema after treated with Na-CMC as control, sodium diclofenac, EAN 0.05, EAN 0.1 and EAN 0.2
***Significantly differences (p<0.05 and p=0.000)

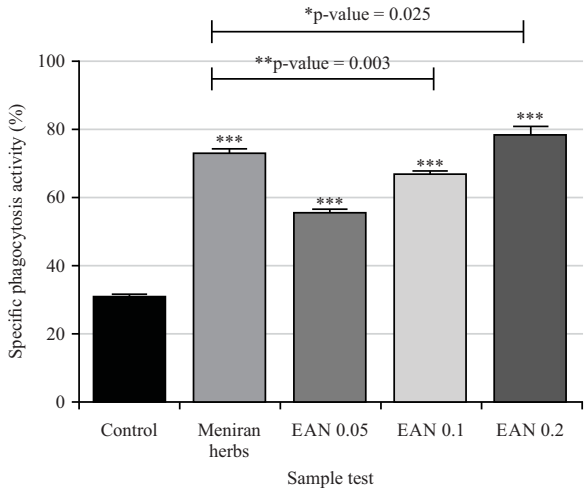

Fig. 3: Phagocytic activity of macrophage in each group
Data is presented as mean±SD

positive control, EAN 0.05, EAN 0.1 and EAN 0.2 were 29.75±0.01, 71.5±0.03, 55.5±0.03, 66.25±0.04 and 77.25±0.04%, respectively.

The sample's immunomodulatory activity was dose-dependent, providing the highest activity with the highest concentration. All doses differed significantly from the positive control and the concentration at 0.2 had a higher percentage of phagocytic activity than the control ($p<0.05$).

DISCUSSION

Nephthea sp. (Nephtheidae) is a soft coral found in Waworaha, Southeast Sulawesi. It is widely distributed in the Indo-Pacific Region, the Islands of Mauritius, Rodrigues and the Brazilian Coast[5]. Soft corals from the Nephtheidae family have attracted attention because they have been shown to contain compounds such as steroids, terpenoids, isoprenoids, nonisoprenoids, quinones, halogenated compounds, nitrogen heterocyclics and sulfur nitrogen heterocyclics which provide many pharmacological activities[18-20]. According to the previous study, the nonpolar subfraction of the ethyl acetate fraction of *Nephthea* sp., has consisted of acilin, atractylenolide II, butyl isobutyl phthalate, rengyolester, 2a-acetoxycostic acid, ocotillol acetate, petasitolone and several compounds that have not been identified. In contrast, in the polar subfraction, the ethyl acetate fraction consisted of 3-ter-butyl-4-methoxyphenol, isosol, valine and some chemicals that have not been identified[5,7]. This study found that *Nephthea* sp., positively contains alkaloids, flavonoids and steroids.

Several pharmacological activities were reported from *Nephthea* sp., including anti-inflammatory, cytotoxic, antidiabetic, antifouling, antibacterial and antiviral[6,20,21]. Current findings suggested that *Nephthea* sp., provides anti-inflammatory activity and immunomodulatory effects. The EAN 0.2 provides the highest anti-inflammatory and immunomodulatory. The inflammatory process usually involves several types of cells, such as neutrophils, basophils, eosinophils, monocytes, macrophages, NK cells, dendritic cells (DCs), T cells and B cells. Several pro and anti-inflammatory mediators are produced, such as cytokines, chemokines, Tumors Necrosis Factor-Alpha (TNF-α) and inducible enzymes (Cyclooxygenase-2 (COX-2) and NO Synthase (iNOS). The COX-2 and iNOX produce proinflammatory prostaglandins. The COX-2 and iNOS inhibitors show potential effects on treating inflammatory diseases[22,23]. According to several studies, soft coral significantly inhibited the accumulation of proinflammatory protein iNOS and effectively reduced the accumulation of COX-2 protein in LPS-stimulated RAW264.7 cells[18]. Other studies have shown that the content of sesquiterpenes can increase the high COX-2 inhibitory activity[12].

Flavonoid compounds have an anti-inflammatory mechanism because they downregulate the expression of iNOS and COX-2 in various inflammatory diseases[24]. In addition, it decreases the production of proinflammatory cytokines/chemokines and the expression levels of MHC class II and costimulatory molecules. In addition, activation of nuclear transcription factor kappa β (NF-kappa β) modulates the expression of Th2 cytokines, including IL-4 and IL-5. These cytokines change the IgE class and suppress the degranulation/secretion of various chemical mediators (PGD2, mMCPT-1 Cys-L and TSLP) from activated mast cells[25]. The alkaloid compounds work by showing inhibitory effects on iNOS and COX-2 activity, reducing NO and PGE2 levels, reducing IL-1β and TNF-α production in LPS-stimulated RAW 264.7 cells, reducing inflammatory infiltrates and levels of proinflammatory cytokines IL-6, IL-12, TNF-, NF-B p65 and IFN-γ and increase levels of the anti-inflammatory cytokine IL-10[26]. The results of this study indicated that *Nephthea* sp., has potential as an anti-inflammatory and immunomodulator.

CONCLUSION

Ethyl acetate extract of *Nephthea* sp., contains secondary metabolites, including flavonoids, alkaloids and steroids. These compounds might contribute to their anti-inflammatory activity by decreasing the edema volume in the ear and immunomodulatory activity. The results of this study can be used as scientific information for the development of *Nephthea* sp., as an anti-inflammatory and immunomodulatory

SIGNIFICANCE STATEMENT

The purpose of this study is to evaluate the anti-inflammatory and immunomodulatory activity of *Nephthea* sp., ethanolic extract from Southeast Sulawesi. Current finding suggested that *Nephthea* sp., ethanolic extract has anti-inflammatory activity in xylene-induce edema in mice' ear, as well as immunomodulatory activity by inducing the specific phagocytic activity (SPA) of macrophages. This finding can be utilized and developed for further study, for example in developing a novel anti-inflammatory and immunomodulatory agent.

ACKNOWLEDGMENT

The authors would like to thank Ministry of Education, Culture, Research and Technology of the Republic of Indonesia for the Research Grant with scheme Penelitian Dasar Unggulan Perguruan Tinggi 2023, Contract No. 28/UN29.20/PG/2023 and 019/E5/PG.02.00.PL/2023

REFERENCES

1. Tanod, W.A., A.T. Aristawati, M.Y. Putra and M. Muliadin, 2020. Soft coral (*Sinularia* sp.) extracts with antibacterial activity. Omni-Akuatika, 14: 108-117.
2. Hanif, N., A. Murni, C. Tanaka and J. Tanaka, 2019. Marine natural products from Indonesian waters. Mar. Drugs, Vol. 17. 10.3390/md17060364.
3. Reverter, M., M. Jackson, S. Rohde, M. Moeller and R. Bara *et al*., 2021. High taxonomic resolution surveys and trait-based analyses reveal multiple benthic regimes in North Sulawesi (Indonesia). Sci. Rep., Vol. 11. 10.1038/s41598-021-95905-8.
4. Dewanto, D.K., Finarti, R. Hermawan, S. Ndobe, P.H. Riyadi and W.A. Tanod, 2019. Antioxidant activity of soft corals extracts from Palu Bay, Central Sulawesi, Indonesia [In Indonesian]. J. Pascapanen Bioteknologi Kelautan Perikanan, 14: 163-178.
5. Sadarun, B., N.S. Rahmatika, A.W.M. Yodha, A. Fristiohady, A. Sundowo, S.N. Baharum and I. Sahidin, 2022. Antioxidant and cytotoxic properties of soft coral *Nepthea* sp. Indones. J. Mar. Sci., 27: 29-36.
6. Abdelhafez, O.H., J.R. Fahim, R.R. El Masri, M.A. Salem and S.Y. Desoukey *et al*., 2021. Chemical and biological studies on the soft coral *Nephthea* sp. RSC Adv., 11: 23654-23663.
7. Wang, S.K., S.Y. Puu and C.Y. Duh, 2013. New steroids from the soft coral *Nephthea chabrolii*. Mar. Drugs, 11: 571-580.
8. Li, B.X., K. Yamanaka and X. Xiao, 2012. Structure-activity relationship studies of naphthol AS-E and its derivatives as anticancer agents by inhibiting CREB-mediated gene transcription. Bioorg. Med. Chem., 20: 6811-6820.
9. Langi, P., A. Yudistira and K.L.R. Mansauda, 2020. Antioxidant activity test of soft coral (*Nepthea* sp.) using the dpph method (1,1-diphenyl-2-picrylhydrazyl) [In Indonesian]. Pharmacon, 9: 425-431.
10. Hsiao, T.H., C.S. Sung, Y.H. Lan, Y.C. Wang and M.C. Lu *et al*., 2015. New anti-inflammatory cembranes from the cultured soft coral *Nephthea columnaris*. Mar. Drugs, 13: 3443-3453.
11. Hsiao, T.H., C.H. Cheng, T.Y. Wu, M.C. Lu and W.F. Chen *et al*., 2015. New cembranoid diterpenes from the cultured octocoral *Nephthea columnaris*. Molecules, 20: 13205-13215.
12. Abdelhafez, O.H., T.F.S. Ali, J.R. Fahim, S.Y. Desoukey and S. Ahmed *et al*., 2020. Anti-inflammatory potential of green synthesized silver nanoparticles of the soft coral *Nephthea* sp. supported by metabolomics analysis and docking studies. Int. J. Nanomed., 15: 5345-5360.
13. Ahmad, B., M. Shah and S. Choi, 2019. Oceans as a source of immunotherapy. Mar. Drugs, Vol. 17. 10.3390/md17050282.
14. Singsai, K., P. Charoongchit, W. Chaikaew, N. Boonma, P. Fhanjaksai and K. Chaisatan, 2020. Antilipoxygenase and anti-inflammatory activities of *Streblus asper* leaf extract on xylene-induced ear edema in mice. Adv. Pharmacol. Pharm. Sci., Vol. 2020. 10.1155/2020/3176391.
15. Cheekavolu, C. and M. Muniappan, 2016. *In vivo* and *in vitro* anti-inflammatory activity of indazole and its derivatives. J. Clin. Diagn. Res., 10: FF01-FF06.
16. Marusin, S. and Chairul, 2012. The effect of water and alcohol extracts on Miswak (*Salvadora persica* L.) on increasing the activity and phagocytic capacity of macrophage cells. Health Res. Dev. Media, 22: 38-44.
17. Saputri, F.C. and R. Zahara, 2016. Anti-inflammatory activity test of basil leaf essential oil (*Ocimum americanum* L.) in male white rats induced by carrageenan [In Indonesian]. Pharm. Sci. Res., 3: 107-119.
18. Fang, H.Y., C.H. Hsu, C.H. Chao, Z.H. Wen, Y.C. Wu, C.F. Dai and J.H. Sheu, 2013. Cytotoxic and anti-inflammatory metabolites from the soft coral *Scleronephthya gracillimum*. Mar. Drugs, 11: 1853-1865.
19. Elshamy, A.I., M.I. Nassar, T.A. Mohamed and M.E.F. Hegazy, 2016. Chemical and biological profile of *Cespitularia* species: A mini review. J. Adv. Res., 7: 209-224.
20. Abdelhafez, O.H., J.R. Fahim, M. Mustafa, A.M. AboulMagd and S.Y. Desoukey *et al*., 2022. Natural metabolites from the soft coral *Nephthea* sp. as potential SARS-CoV-2 main protease inhibitors. Nat. Prod. Res., 36: 2893-2896.
21. Ermolenko, E.V., A.B. Imbs, T.A. Gloriozova, V.V. Poroikov, T.V. Sikorskaya and V.M. Dembitsky, 2020. Chemical diversity of soft coral steroids and their pharmacological activities. Mar. Drugs, Vol. 18. 10.3390/md18120613.
22. Boroumand, N., S. Samarghandian and S.I. Hashemy, 2018. Immunomodulatory, anti-inflammatory, and antioxidant effects of curcumin. J. Herbmed. Pharmacol., 7: 211-219.
23. Lin, C.Y., M.C Lu, J.H. Su, C.L. Chu and D. Shiuan *et al*., 2013. Immunomodulatory effect of marine cembrane-type diterpenoids on dendritic cells. Mar. Drugs, 11: 1336-1350.
24. González-Gallego, J., M.V. García-Mediavilla, S. Sánchez-Campos and M.J. Tuñón, 2013. Anti-Inflammatory and Immunomodulatory Properties of Dietary Flavonoids. In: Polyphenols in Human Health and Disease, Watson, R.R., V.R. Preedy and S. Zibadi (Eds.), Academic Press, United States, ISBN: 978-0-12-398456-2, pp: 435-452.
25. Hosseinzade, A., O. Sadeghi, A.N. Biregani, S. Soukhtehzari, G.S. Brandt and A. Esmaillzadeh, 2019. Immunomodulatory effects of flavonoids: Possible induction of T CD4+ regulatory cells through suppression of mTOR pathway signaling activity. Front. Immunol., Vol. 10. 10.3389/fimmu.2019.00051.
26. Souza, C.R.M., W.P. Bezerra and J.T. Souto, 2020. Marine alkaloids with anti-inflammatory activity: Current knowledge and future perspectives. Mar. Drugs, Vol. 18. 10.3390/md18030147.