

# Extraction and Fractionation of the Seaweed *Sargassum plagyophyllum* and Evaluation of Fractions on Depression Induced by Interferon Alpha in Mice

## Abstract

**Background:** Marine organisms such as seaweeds, produce potent chemicals with characteristic biological features. *Sargassum* species have great potential to be used for neuronal protection as part of nutraceuticals. The aim was to investigate the effects of hexane and methanol extracts of *Sargassum plagyophyllum* from the Persian Gulf on depression induced by interferon- $\alpha$  (IFN $\alpha$ ) in mice. **Materials and Methods:** *S. plagyophyllum* was extracted by maceration with methanol-ethyl acetate solvent (1:1). The extract was evaporated and partitioned by hexane and methanol solvents. Male mice were used, depression was induced by SC injecting IFN $\alpha$  ( $16 \times 10^5$  IU/kg) for 6 days. Animals were subject to the forced swimming test (FST) after the locomotor test, on day 7. The extracts were administered IP either one single dose (acute) before the test, or simultaneously with IFN $\alpha$  (sub-acute). **Results:** The locomotor activity was not different from control values. IFN $\alpha$  increased the immobility time during FST ( $140 \pm 14$  s vs. control group  $95 \pm 9$  s,  $P < 0.05$ ). Hexane extract acute (40 mg/kg) injection was not effective while its sub-acute (20 mg/kg) injection reduced immobility time ( $46 \pm 8$  s,  $P < 0.001$  vs. IFN $\alpha$  alone). Methanol extract acute (20 mg/kg) and sub-acute (20 mg/kg) administration significantly reduced immobility during the FST ( $78 \pm 20$  s, and  $72 \pm 8$  s respectively,  $P < 0.05$  vs. IFN $\alpha$  alone). **Conclusion:** *S. plagyophyllum* has antidepressant effects, the hexane extract could prevent depression while the methanol extract not only prevented but also treated depression induced by IFN $\alpha$  in mice. Since this species is abundant in the Persian Gulf further clinical studies on its psychological effects are warranted.

**Keywords:** Depression, Interferon-alpha, *Sargassum plagyophyllum*, seaweed

## Introduction

Depression is one of the most prevalent chronic and disabling psychological problems leading to high morbidity and mortality.<sup>[1]</sup> Apart from the monoamine hypothesis of depression cytokines are another leading cause of depression.<sup>[2]</sup> Interferon- $\alpha$  (IFN $\alpha$ ) is a cytokine with important role in innate immunity against viral infections, therapeutically it is used for hepatitis C and various types of cancer.<sup>[3]</sup> IFN- $\alpha$  side effects generally induce “flu-like” symptoms; in addition, it can induce neuropsychiatric side effects notably depression symptoms within 2–3 months of usage (Lotrich, 2009). IFN $\alpha$  increases indoleamine 2,3-dioxygenase (IDO) enzyme activity; which converts tryptophan to kynurenine. Therefore, following IFN $\alpha$  administration by activating IDO kynurenine increases on

the other hand the available concentration of tryptophan required for serotonin synthesis, decreases.<sup>[4]</sup>

Based on the monoamine hypothesis of depression different types of antidepressant drugs are used in the clinic, but the most important problem is the side effects such as sexual dysfunction, coronary heart disease, and fracture risk usually reported with the use of these antidepressants.<sup>[5,6]</sup> Therefore, natural products with fewer side effects than chemical ones, have become an alternative to modern antidepressants.

Marine habitat is an important source of biologically active metabolites. Isolated compounds from marine organisms are different from terrestrial natural metabolites due to the unique physical and chemical conditions in marine litter. Hence, marine organisms such as seaweeds, sponges, corals, fungi, and ascidians produce potent

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow\_reprints@wolterskluwer.com

**How to cite this article:** Mesripour A, Gholamzadeh E, Yegdaneh A. Extraction and fractionation of the seaweed *Sargassum plagyophyllum* and evaluation of fractions on depression induced by interferon alpha in mice. *Adv Biomed Res* 2022;11:59.

Azadeh Mesripour,  
Ehsan  
Gholamzadeh<sup>1</sup>,  
Afsaneh Yegdaneh<sup>1</sup>

Departments of Pharmacology  
and <sup>1</sup>Pharmacognosy, Isfahan  
Pharmaceutical Sciences  
Research Center, School of  
Pharmacy and Pharmaceutical  
Sciences, Isfahan University of  
Medical Sciences, Isfahan, Iran

### Address for correspondence:

Dr. Afsaneh Yegdaneh,  
Department of Pharmacognosy,  
Isfahan Pharmaceutical Sciences  
Research Center, School of  
Pharmacy and Pharmaceutical  
Sciences, Isfahan University of  
Medical Sciences, Isfahan, Iran.  
E-mail: yekdaneh@pharm.mui.  
ac.ir

Received: 26 June 2021

Revised: 03 August 2021

Accepted: 25 October 2021

Published: 29 July 2022

### Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr\_186\_21

### Quick Response Code:



chemicals with characteristic structural and biological features. In the field of marine plants, more than 2400 marine natural compounds have been isolated only from seaweeds of subtropical and tropical populations.<sup>[7]</sup> Researchers have reported that algal-originated compounds exhibit various biological activities such as anticoagulant, anti-viral, antioxidant, anti-allergic, anti-cancer, anti-inflammatory, and anti-obesity.<sup>[8-10]</sup> Furthermore, several scientific studies have provided insight into the neuroprotective properties of marine algae.

*Sargassum* is a genus of brown (class Phaeophyceae) macroalgae (seaweed) in the order Fucales. Numerous species are distributed throughout the temperate and tropical oceans of the world. Recently, several scientific studies have provided an insight into biological activities and neuroprotective effects of *Sargassum* species including antioxidant, anti-neuroinflammatory, cholinesterase inhibitory activity, and the inhibition of neuronal death suggesting that this algae have great potential to be used for neuroprotection as part of pharmaceuticals, nutraceuticals, and functional foods.<sup>[11-13]</sup>

IFNa has multiple uses in the clinic that may cause depression in patients, however depression prophylaxis with antidepressant drugs may unnecessarily imposed individuals to polypharmacy. The main aim of the present study was to initially fractionate the seaweed *Sargassum plagyophyllum* and to evaluate the effect of fractions on depression-like behavior induced by IFNa in mice for the first time.

## Materials and Methods

### Authentication of plant material

*S. plagyophyllum* was collected from Bushehr, a Southwest coastline of the Persian Gulf, in 2015. Voucher specimens were made and identified by the Agricultural and Natural Resources Research Center of Bushehr (Code: 2662).

### Preparation of the extracts

The algae were dried at room temperature in the shade and ground to powder, then extracted by maceration with methanol-ethyl acetate (1:1) at room temperature. The extraction method was repeated four times and the solvent was evaporated under vacuum and partitioned to yield Hexane and methanol partitions. The partitions were subjected to the antidepressant test.

### Animals

Male albino mice weighing  $22 \pm 3$  g were maintained at room temperature  $21^\circ\text{C} \pm 2^\circ\text{C}$  with free access to standard mice chow and tap-water, on a 12–12 h light-dark cycle (lights on at 6 AM). Six animals were housed together in a cage, and they were placed in the experimental room 24 h before the test for acclimatization. All the experiments were performed between 8 AM and 1

PM in the pharmacology laboratory. All animal procedures were performed in accordance with guidelines for the Care and Use of Laboratory Animals Issued by The National Ethical Committee (Ethical No: IR.MUI.REC.1398.041). All the efforts in the experiments were made to minimize animal suffering and to reduce the number of animals used in the experiments.

### Locomotor test

The motor activity of mice was assessed before the forced swimming test (FST) in an open arena (Borj Sanat, Iran) divided into 15 zones by red beams. Mice were allowed to explore the field for 3 min, by passing through the beams the number of zone entries was counted automatically while rears on hind legs were recorded manually. Finally, total activity for each animal was calculated which was the sum of zone entries (horizontal exploration) and rears (vertical exploration).

### Forced swimming test

This test was performed as an animal model of despair behavior. Mice were forced to swim in  $25^\circ\text{C}$  water in a glass 2-L beaker (diameter 12.5 cm, depth 12 cm) for 6 min. The immobility time defined when no additional activity was observed other than that required to keep the animals' head above the water was measured during the last 4 min of the trial after habituation was considered at the first 2 min. Swimming behavior, defined as horizontal movement throughout the beaker which involved at least two limbs; and, climbing behavior, defined as upward movements of the forepaws along the side of the beaker were also recorded.<sup>[14]</sup> The whole experiment was recorded by a camera and analyzed later. After 6 min, the mice were dried carefully to avoid hypothermia and returned to their home cage. Animals were subject to FST after the locomotor test.

### Drugs and *Sargassum plagyophyllum* extracts administration

IFNa (PDferon, Pooyesh Darou  $3 \times 10^6$  IU, Iran)  $16 \times 10^5$  IU/kg body weight was injected SC for 6 consecutive days, the tests were performed on the following day (Fashi *et al.*, 2017). Fluoxetine HCl (a gift from Pars Daru, Iran) 20 mg/kg was injected IP 30 min prior to the tests as the reference antidepressant drug. The methanol and hexane extract (20 and 40 mg/kg) were administered IP. The methanol extract was dilution in normal saline, thus the vehicle group received normal saline. The hexane extract was diluted in 0.1% tween 80 (Merck Germany) in normal saline and the vehicle group received 0.1% tween 80 in normal saline.

To find the best dose of the extracts they were first administered alone and the test was performed after an hour. The extracts were then administered either after depression was induced by IFNa on day 7 (acute,

40 mg/kg of each extract) or they were injected together with IFNa (sub-acute, 20 mg/kg of each extract) for 6 days and the test was performed on the following day.

### Data processing and statistical analysis

Results were expressed as group mean  $\pm$  standard error of mean. All results were analyzed using one-way analysis of variance, followed by Tukey's multiple comparison tests.  $P < 0.05$  were considered significant. The software programs used for data analyzing and making graphs were Excel 2010 and the GraphPad Prizm 6 (GraphPad Software, Inc., San Diego, California, USA).

## Results

### The effects of interferon- $\alpha$ and *Sargassum plagyophylum* extracts on the locomotor activity

The locomotor activity should be performed before behavioral tests because variations in animal locomotor activity nonspecifically affect actions in many behavioral tests. As it is shown in Table 1, there was no significant difference in locomotor activity between different groups. Therefore, changes in the immobility time observed during the FST could be deduced as depressive behavior. Acute refers to, 40 mg/kg of each extract administered after depression was induced by IFNa on day 7. Sub-acute refers to, 20 mg/kg of each extract that was injected together with IFNa for 6 days and the test was performed on the following day.

### The effect of *Sargassum plagyophylum* hexane extract on the forced swimming test

As it is shown in Figure 1a, IFNa significantly increased the immobility time during FST ( $140 \pm 14$  s vs. control group  $95 \pm 9$  s,  $P < 0.05$ ) that clearly showed that depression was induced in the animals. Table 2 shows that IFNa has reduced the swimming time and climbing time but the value was only significant for the climbing

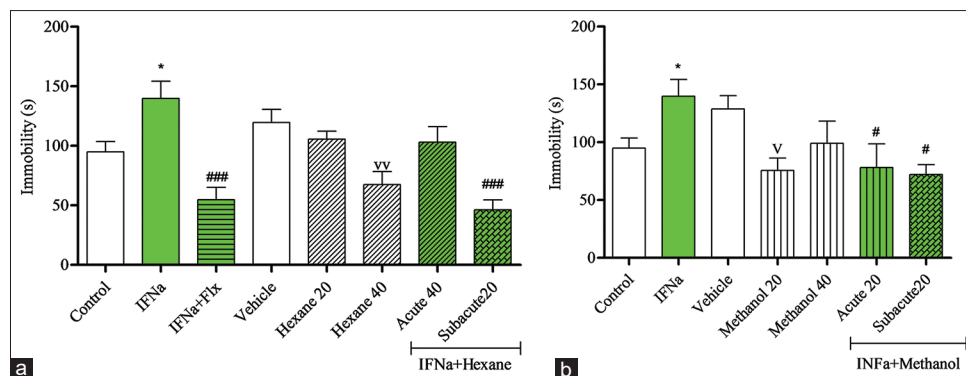
time ( $P < 0.05$ ). Fluoxetine used as the reference drug on day 7 following IFNa administration before the test decreased the immobility time to  $55 \pm 10$  s [ $P < 0.001$  vs. IFNa group, Figure 1a]. As shown in Table 2, fluoxetine significantly increased the swimming time ( $P < 0.05$  vs. IFNa group), while higher climbing time was not noticeable.

Presented in Figure 1a, the hexane extract alone in its highest dose (40 mg/kg) significantly reduced the immobility time in the FST ( $67 \pm 11$  s vs. vehicle group  $120 \pm 11$  s,  $P < 0.01$ ), but this did not happen when the animals were depressed by IFNa. The hexane extract (40 mg/kg) increased swimming time and climbing time although the values did not reach significant levels [Table 2]. Administrating the low dose hexane extract for 6 days (sub-acute) together with IFNa prevented the depression behavior ( $46 \pm 8$  s,  $P < 0.001$  vs. IFNa group). Swimming and climbing time was also higher than IFNa

**Table 1: Effect of the hexane extract on activities during forced swimming test**

Group	FST	
	Swimming (s)	Climbing (s)
Control	122 $\pm$ 7	25 $\pm$ 4
IFNa	93 $\pm$ 17	8 $\pm$ 3*
IFNa + Flx	170 $\pm$ 12 <sup>#</sup>	21 $\pm$ 7
Vehicle	114 $\pm$ 15	10 $\pm$ 6
Hexane 20 mg/kg	114 $\pm$ 8	20 $\pm$ 7
Hexane 40 mg/kg	145 $\pm$ 6	32 $\pm$ 4
IFNa + acute hexane	142 $\pm$ 16	13 $\pm$ 4
IFNa + sub-acute hexane	166 $\pm$ 15 <sup>#</sup>	21 $\pm$ 8

\* $P < 0.05$  compared with the control versus  $P < 0.05$  compared with the vehicle, <sup>#</sup> $P < 0.05$ , compared with the IFNa alone. The control and vehicle groups received normal saline and 0.1% tween 80/normal saline, respectively. Results are expressed as group mean $\pm$ SEM and analyzed by ANOVA followed by Tukey's comparison test ( $n=6$ ). ANOVA: Analysis of variance, SEM: Standard error of the mean, IFNa: Interferon- $\alpha$ , FST: Forced swimming test, Flx: Fluoxetine



**Figure 1: The effect of *Sargassum plagyophylum* extracts on the immobility time during the last 4 min of the forced swimming test. (a) the immobility time related to the hexane extract and (b) is related to the methanol extract. Interferon- $\alpha$  was injected SC  $16 \times 10^5$  IU/kg for 6 days control group received normal saline. The methanol and hexane extracts were injected in IP (20, 40 mg/kg), the methanol vehicle group received normal saline, and the hexane vehicle group received 0.1% tween 80 in normal saline. The number of animals in each group was 6. Results are expressed as group mean  $\pm$  standard error of mean and analyzed by analysis of variance followed by Tukey's comparison tests. \* $P < 0.05$ , compared with control group, <sup>V</sup> $P < 0.05$  compared with vehicle group, <sup>#</sup> $P < 0.05$  compared with interferon- $\alpha$  alone group**

**Table 2: Effect of the methanol extract on activities during forced swimming test**

Group	FST	
	Swimming (s)	Climbing (s)
Control	122±7	25±4
IFNa	93±17	8±3*
IFNa+Flx	170±12 <sup>#</sup>	21±7
Vehicle	100±7	13±5
Methanol 20 mg/kg	137±7 <sup>v</sup>	7±2
Methanol 40 mg/kg	122±15	20±8
IFNa + acute methanol	161±20 <sup>#</sup>	7±3
IFNa + sub-acute methanol	161±10 <sup>#</sup>	6.5±4

\* $P < 0.05$  compared with the control versus  $P < 0.05$  compared with the vehicle, <sup>#</sup> $P < 0.05$ , compared with the IFNa alone. The control group received normal saline, methanol extract was diluted in normal saline, the vehicle group received normal saline. Results are expressed as group mean±SEM and analyzed by ANOVA followed by Tukey's comparison test ( $n=6$ ). ANOVA: Analysis of variance, SEM: Standard error of the mean, IFNa: Interferon- $\alpha$ , FST: Forced swimming test, Flx: Fluoxetine

alone group although only swimming time was significantly higher ( $P < 0.05$  vs. IFNa group).

### The effect of *Sargassum plagyophylum* methanol extract on the forced swimming test

As it is shown in Figure 1b, the methanol extract reduced the immobility time, while it was only significant for the lower dose (20 mg/kg,  $72 \pm 12$  s  $P < 0.05$  vs. vehicle group  $121 \pm 9$  s). The lower methanol dose also significantly increased the swimming time [ $P < 0.05$  vs. vehicle group, Table 2]. Methanol extract could also reduce the immobility time following IFNa administration, not only when it was injected together with IFNa (sub-acute;  $72 \pm 8$  s,  $P < 0.05$  vs. IFNa group) but also when it was injection on the final day after IFNa (acute;  $78 \pm 20$  s,  $P < 0.05$  vs. IFNa group), thus it proved antidepressant effects. On the other hand, swimming time was also higher than the TNFa group for both acute and sub-acute treatments with methanol ( $P < 0.05$ ), while the climbing times were indifferent [Table 2].

The vehicle groups of hexane and methanol were merged together. Acute refers to, 40 mg/kg of each extract administered after depression was induced by IFNa on day 7. Sub-acute refers to, 20 mg/kg of each extract that was injected together with IFNa for 6 days and the test was performed on the following day. \* $P < 0.05$  compared with control group; v,  $P < 0.05$  compared with vehicle group; #,  $P < 0.05$  compared with IFNa group;

## Discussion

Our results for the first time showed that *S. plagyophylum* hexane and methanol extracts could prevent IFNa induced depression in mice. A single methanol extract administration could treat IFNa depression, while the hexane extract only prevented this effect. The doses were adjusted so that no

important change would happen in animals' locomotor activity. As the reference antidepressant drug, fluoxetine reduced the immobility time during FST it verified the validity of our experimental condition. The FST is the most widely used paradigm to evaluate depression-and antidepressant-like behavior, also known as Porsolt's test.<sup>[15]</sup> The FST is easily handled and has great reliability across laboratories. It takes advantage of a rodent behavior that, after initial escape-attempt movements, rapidly in an inescapable water-filled cylinder adopt a characteristic immobile posture, which is interpreted as behavioral despair that is a depression-like behavior. Interestingly, the quantity of different movements that is climbing and swimming behavior has predictive value to differentiate between catecholaminergic and serotonergic substances, respectively.<sup>[16]</sup>

IFNa administration for 6 days as it was observed earlier increased the immobility time that clearly indicated the induction of depression.<sup>[17]</sup> It was proven previously that *S. plagyophylum* hexane and methanol extract decreases immobility in the FST,<sup>[18]</sup> in the following study we extended the findings on the possible antidepressant effects of *S. plagyophylum* extracts by inducing depression by IFNa. The glutamate system and cytokines are closely related through the tryptophan–kynurenine pathway with vital role in depression pathophysiology.<sup>[19]</sup> Tryptophan either converts to serotonin or by IDO activation it converts to kynurenine. Kynurenine is then metabolized to quinolinic acid that is an agonist of glutamate receptor; N-methyl-D-aspartate (NMDA). Immoderate NMDA receptor stimulation causes neuronal excitotoxic damage.<sup>[19]</sup> IFN $\alpha$  induces this pathway by activating IDO and increasing the quinolinic acid/serotonin ratio, which might be related to depression.<sup>[20]</sup>

*S. plagyophylum* hexane extract higher dose (40 mg/kg) reduced the immobility time in the FST, which proved its antidepressant-like effects. Swimming and climbing were both increased although the values were not increased significantly. This might indicate that the hexane extract could influence both noradrenaline and serotonergic neurotransmitters. It was previously shown that fucosterol, a sterol compound of *S. fusiforme*, considerably reduced the immobility time of mice during the tail suspension test and FST.<sup>[21]</sup> It was deduced that this effect of fucosterol is likely mediated by an increase in central nervous system (CNS) serotonin and noradrenaline concentrations. One of the dominant components of human cell membranes and constituent substances in the CNS is cholesterol.<sup>[22]</sup> Evidence also advocates steroids antidepressant activity.<sup>[23]</sup> Steroids present in the hexane extract could be a good reason for its antidepressant effects in our study during the FST. By injecting the hexane extract after IFNa-induced depression, the extract did not noticeably reduce the immobility time. On the other hand, when it was administered together with

IFNa for 6 days (sub-acute) it profoundly reduced the immobility time during FST, while the swimming and climbing also increased but the value was only noticeable for the swimming time. Therefore it was interpreted that the hexane extract single dose could not treat depression induced by IFNa, but it could prevent IFNa induced depression. Since the swimming behavior was also increased by the sub-acute injections of the extract, that was similar changes observed when fluoxetine was administered, therefore the serotonergic system may have been dominant in the antidepressant effects. That is to say, possibly the extract has prevented the imbalance between serotonin and kynurenine production that was the direct effect of IDO induction by IFNa. This would be an interesting area for further studies regarding the effect of *S. plagyophylum* extract on IDO activity and the serotonin, kynurenine, and related metabolites concentration *in-vivo* and *in-vitro*.

In agreement with previous studies, the methanol extract showed antidepressant effects in its lower dose while increasing the swimming time.<sup>[18]</sup> *Sargassum* species are rich sources of bioactive compounds such as vitamins, dietary fibers, proteins, carotenoids, and minerals.<sup>[7]</sup> The role of diet, nutrition, and minerals, on mental health and their relation to depression are becoming fascinating.<sup>[24,25]</sup> At least in part, the antidepressant effects of the methanol extract could be related to its vitamins and nutritional composition. The acute and sub-acute methanol extract administration reduced the immobility time during FST. It was interpreted that the extract not only prevented IFNa induced depression but also could treat it by a single dose. The swimming time was significantly increased that could be thought as the effect of methanol extract on the serotonin level, which warrants further evaluations. Different mechanisms could have been involved in the antidepressant effects of acute and sub-acute methanol extract administration following IFNa injection. It was previously reported that the methanol extract of *S. Macrocarpum* with the active purified substance sargachromenol has marked nerve growth factor (NGF)-dependent neurite outgrowth stimulating effect on PC12D cells, but it does not induce neural differentiation in the absence of NGF.<sup>[26]</sup>

## Conclusion

The hexane extract could prevent depression induced by IFNa while the methanol extract not only prevented but also treated depression induced by IFNa in the mice model of despair. As previously mentioned IFNa can augment IDO enzyme activity that leads to an increase in kynurenine and a decrease in available tryptophan required for serotonin synthesis.<sup>[27]</sup> Interestingly the swimming behavior was increased during the active phase of the FST that is proven to be related to the serotonergic system. Therefore, it is suggested to measure IDO activity following applying different extracts of *S. plagyophylum* and also measuring

the norepinephrine, serotonin, and their metabolites level in the brain and serum.

Kynurenine crosses the blood–brain barrier and it converts to neurotoxic substances such as quinolinic acid that is an agonist of NMDA.<sup>[27]</sup>

## Financial support and sponsorship

The authors are thankful to the Vice Chancellor of Research, Isfahan University of Medical Sciences and Iran National Science Foundation (INSF) for financial support. The results described in this paper are part of a Pharm.D thesis.

## Conflicts of interest

There are no conflicts of interest.

## References

- Gaffrey MS, Luby JL, Barch DM. Towards the study of functional brain development in depression: An interactive specialization approach. *Neurobiol Dis* 2013;52:38-48.
- Maes M. The cytokine hypothesis of depression: Inflammation, oxidative and nitrosative stress (IO and NS) and leaky gut as new targets for adjunctive treatments in depression. *Neuro Endocrinol Lett* 2008;29:287-91.
- Friedman RM. Clinical uses of interferons. *Br J Clin Pharmacol* 2008;65:158-62.
- Baranyi A, Meinitzer A, Breitenecker RJ, Amouzadeh-Ghadikolai O, Stauber R, Rothenhäusler HB. Quinolinic acid responses during interferon- $\alpha$ -induced depressive symptomatology in patients with chronic hepatitis C infection – A novel aspect for depression and inflammatory hypothesis. *PLoS One* 2015;10:e0137022.
- Eom CS, Lee HK, Ye S, Park SM, Cho KH. Use of selective serotonin reuptake inhibitors and risk of fracture: A systematic review and meta-analysis. *J Bone Miner Res* 2012;27:1186-95.
- Fournier JC, DeRubeis RJ, Hollon SD, Dimidjian S, Amsterdam JD, Shelton RC, *et al.* Antidepressant drug effects and depression severity: A patient-level meta-analysis. *JAMA* 2010;303:47-53.
- Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep* 2009;26:170-244.
- Yegdaneh A, Ghannadi A, Dayani L. Chemical constituents and biological activities of two Iranian *Cystoseira* species. *Res Pharm Sci* 2016;11:311-7.
- Tang HF, Yang-Hua Y, Yao XS, Xu QZ, Zhang SY, Lin HW. Bioactive steroids from the brown alga *Sargassum carpophyllum*. *J Asian Nat Prod Res* 2002;4:95-101.
- Mehdinezhad N, Ghannadi A, Yegdaneh A. Phytochemical and biological evaluation of some *Sargassum* species from Persian Gulf. *Res Pharm Sci* 2016;11:243-9.
- Ina A, Hayashi K, Nozaki H, Kamei Y. Pheophytin a, a low molecular weight compound found in the marine brown alga *Sargassum fulvellum*, promotes the differentiation of PC12 cells. *Int J Dev Neurosci* 2007;25:63-8.
- Ahn BR, Moon HE, Kim HR, Jung HA, Choi JS. Neuroprotective effect of edible brown alga *Eisenia bicyclis* on amyloid beta peptide-induced toxicity in PC12 cells. *Arch Pharm Res* 2012;35:1989-98.
- Neto MR, Tintino SR, da Silva AR, Costa MD, Boligon AA,

- Matias EF, *et al.* Seasonal variation of Brazilian red propolis: Antibacterial activity, synergistic effect and phytochemical screening. *Food Chem Toxicol* 2017;107:572-80.
14. Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol* 1997;8:523-32.
  15. Deussing JM. Animal models of depression. *Drug Discov Today Dis Model* 2006;3:375-83.
  16. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends Pharmacol Sci* 2002;23:238-45.
  17. Azimi Fashi Y, Mesripour A, Hajhashemi V. Evaluation of the effect of soybean diet on interferon- $\alpha$ -induced depression in male mice. *Avicenna J Phytomed* 2017;7:436-43.
  18. Mesripour A, Rabian N, Yegdaneh A. The effect of different partitions of seaweed *Sargassum plagyophylum* on depression behavior in mice model of despair. *J Complement Integr Med* 2019;16:2007-18.
  19. Müller N, Myint AM, Schwarz MJ. Inflammatory biomarkers and depression. *Neurotox Res* 2011;19:308-18.
  20. Braun D, Longman RS, Albert ML. A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation. *Blood* 2005;106:2375-81.
  21. Zhen XH, Quan YC, Jiang HY, Wen ZS, Qu YL, Guan LP. Fucosterol, a sterol extracted from *Sargassum fusiforme*, shows antidepressant and anticonvulsant effects. *Eur J Pharmacol* 2015;768:131-8.
  22. Korematsu S, Uchiyama SI, Honda A, Izumi T. A new cholesterol biosynthesis and absorption disorder associated with epilepsy, hypogonadism, and cerebro-cerebello-bulbar degeneration. *Pediatr Neurol* 2014;50:601-4.
  23. Machado RA, Espinosa AG, Montoto AP. Cholesterol concentrations and clinical response to sertraline in patients with epilepsy: Preliminary results. *Epilepsy Behav* 2010;19:509-12.
  24. Owen L, Corfe B. The role of diet and nutrition on mental health and wellbeing. *Proc Nutr Soc* 2017;76:425-6.
  25. Williams AL, Cotter A, Sabina A, Girard C, Goodman J, Katz DL. The role for vitamin B-6 as treatment for depression: A systematic review. *Fam Pract* 2005;22:532-7.
  26. Tsang CK, Ina A, Goto T, Kamei Y. Sargachromenol, a novel nerve growth factor-potentiating substance isolated from *Sargassum macrocarpum*, promotes neurite outgrowth and survival via distinct signaling pathways in PC12D cells. *Neuroscience* 2005;132:633-43.
  27. Wichers MC, Koek GH, Robaey G, Verkerk R, Scharpé S, Maes M. IDO and interferon-alpha-induced depressive symptoms: A shift in hypothesis from tryptophan depletion to neurotoxicity. *Mol Psychiatry* 2005;10:538-44.