Indian Journal of Natural Products and Resources
Vol. 12(3), September 2021, pp. 445-450

Bioactive constituents and *in vitro* antibacterial properties of *Petroselinum crispum* leaves, a common food herb in Saudi Arabia

Sivakumar Sivagurunathan Moni[1*], Aamena Jabeen[1], Syeda Sanobar[2], Zia ur Rehman[3], Md Shamsher Alam[3] and Mohamed Eltaib Elmobark[1]

[1]Department of Pharmaceutics, [2]Department of Pharmacognosy, and [3]Department of Pharmaceutical Chemistry, College of Pharmacy, Jazan University, Jazan, Saudi Arabia

Received 18 November 2020; Revised 30 April 2021

Herbs used as daily food additives are good resources for novel pharmaceutical agents. The study investigated the bioactive components of cold methanol maceration extract of *Petroselinum crispum* leaves. Spectral analysis with GC-MS and FT-IR studies demonstrated the presence of fatty acids and steroids, with fatty acids being the predominant components. GC-MS analysis showed the presence of 13-docosenoic acid methyl ester, (Z), cis-13-docosenoic acid, cis-11-eicosenoic acid methyl ester, 11-octadecenoic acid (stearate), methyl ester, hexadecanoic acid (palmitate) methyl ester, 15-tetracosenoic acid methyl ester (Z), cyclopentanone, 3,4-bis(methylene), and stigmastan-3-ol, 5-chloro- acetate. The FT-IR analysis of the fingerprint region displayed significant peaks at 3176.08, 2949, 2173, and 1018 cm^{-1}, indicating the presence of aliphatic amino acids, steroidal compounds, isothiocyanates, polysaccharides, tannins and saponins. The cold methanolic extract (CME) of *P. crispum* produced a low spectrum of antibacterial effects against some screened human pathogenic bacteria and the phytochemical analysis of the extract revealed the presence of carbohydrates, steroids, and saponins. The extract exhibited a better spectrum of activity against Gram-negative bacteria than against Gram-positive bacteria.

Keywords: Alkaloids, Bioactive compounds, Fatty acids, *Petroselinum crispum*, Steroids, Terpenes.

IPC code; Int. cl. (2015.01)-A61K 36/00, A61K 36/23, A61P 31/04

Introduction

The global rise in bacterial resistance threatens the effectiveness of antibiotics regarding the prevention and treatment of infections and the resistance is a major hurdle in the use of antibiotic treatment for bacterial infections[1]. Herb-derived bioactive compounds exhibiting various pharmacological properties can be used as medicines for treating several diseases. The antibacterial efficacy of bioactive molecules of herbal origin has been investigated in the last 3 decades. These studies[1,2] have revealed that herb-derived bioactive compounds are of therapeutical importance in combatting drug resistance. Furthermore, nutrition plays a significant role in maintaining a healthy life. *Petroselinum crispum* belongs to the family Apiaceae and is commonly known as parsley.

In ancient times, the herb was used not only for culinary and medicinal purposes but was also subjected to a variety of superstitious practices by the Greeks and ancient Romans[3]. The roots of parsley have long been used as a diuretic, while the seeds have been used to treat gastrointestinal disorders, diarrhoea, halitosis, kidney stones, inflammation, and amenorrhea[4-6]. Parsley is commonly used as a food additive in Saudi Arabia and has attracted much attention as a basic food additive due to its health benefits[7]. An earlier report showed the importance of *P. crispum* as food owing to its medicinal value[8] An earlier report suggested the medicinal significance of *P. crispum*[9]. However, not much is known about the bioactive compounds and antibacterial properties of the leaves of *P. crispum.* The commercially available samples of the plant in the Jazan market, Saudi Arabia, have not been previously investigated. Thus, the present research work was designed to study the bioactive compounds in the leaves of *P. crispum* which are used as a food additive in Saudi Arabia. A cold methanol extract (CME) of the leaves was subjected to phytochemical screening, GC-MS, FT-IR spectral analysis, and *in vitro* antibacterial activity.

Materials and Methods

Collection and processing of *P. crispum*

A bundle of *P. crispum* was purchased from a local market in Jazan, Saudi Arabia. The herb was

*Correspondent author
Email: drsmsivakumar@gmail.com, smoni@jazanu.edu.sa

immediately transported to the laboratory in an aseptic polyethylene bag. The plant sample was identified by Dr. Remesh Moochikkal, herbarium curator of Jazan University herbarium and a voucher specimen (No. JAZUH 1638) was deposited for future reference. The plant material was thoroughly washed with tap water, followed by washing in Millipore water to remove adherent impurities. It was then air-dried for 30 minutes on a thin polyethylene sheet spread on the floor in a well-ventilated room.

Cold extraction process

The bioactive constituents of the plant were extracted with a cold maceration process, with methanol as the solvent. Fresh leaves of *P. crispum* (100 g) were soaked in 500 mL of methanol for 72 hours. The extract was filtered through Whatman No. 1 filter paper and the filtrate was dried at room temperature. Thereafter, the dried sample was subjected to phytochemical and spectral analysis to determine the presence of bioactive constituents, and its anti-bacterial effects were investigated.

Phytochemical analysis of the extract

The extract was subjected to phytochemical analysis to determine the presence of carbohydrates, proteins, amino acids, tannins, steroids, saponins, flavonoids, and alkaloids, in line with procedures reported earlier[8].

GC-MS analysis

The presence of various bioactive compounds was determined by analyzing the petroleum ether extract using Thermo Scientific GC-MS with AS 3000 autosampler and ISQ detector. The dried powdered sample was diluted in methanol, and 2 µL of the solution was injected into a TR 5MS capillary column for partial separation of the bioactive components. Helium was used as carrier gas at a flow rate of 1.2 mL/min. Mass spectrophotometry was operated and spectral analysis was performed using Xcalibur software. Interpretation of mass spectrum was performed with in-built NIST and MAINLIB software library[2,10].

FT-IR analysis

Leaf powder of *P. crispum* was analyzed using Nicolet iS10 FT-IR spectrophotometer, based on the pressed pellet technique. The spectra of the pellet sample were obtained through FT-IR spectrophotometer against reference KBr pellet in the wavelength range of 400-4000 cm^{-1}, with a resolution of 4 cm^{-1} [Ref. 2,11].

Antibacterial studies[10,11]

Six human pathogenic bacteria comprising equal sets of Gram-positive and Gram-negative bacteria were employed in this study. Initially, subcultures of *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa* were prepared in nutrient broth and incubated at 37 °C for 24 hours. These were designated as 24-h cultures. The 24-h cultures were standardized using serial dilution-agar plate procedure. In this process, the 24-h cultures were diluted serially using sterilized nutrient broth, resulting in a working bacterial culture with a concentration gradient of 10^{-1} to 10^{-9}. The working cultures were spread on agar plates using the pour plate technique, with individual organisms in their respective plates. The potential viability of the working bacterial cultures was determined in terms of colony-forming unit per mL (CFU/mL).

Agar well diffusion technique

A specified quantity of Muller Hinton (MH) agar was dissolved in Millipore water and sterilized in an autoclave. The agar plates were prepared aseptically and the organisms were seeded onto individual MH agar plates using the spread plate technique. The plates were covered with their lids, and the culture was allowed to diffuse through the media for 30 minutes. Wells of diameter 10 mm were made for individual organisms using a sterile standard borer. The extract was added to the wells, and the plates were incubated at 37 °C for 24 hours. The antibacterial effects of CME were quantified by measuring the zone of inhibition around each well in millimetres (mm).

Agar disc diffusion technique

The Kirby–Bauer technique was employed, with standard ciprofloxacin discs (5 µg/disc). The procedure used was similar to that employed in the agar well diffusion technique, but in this case, ciprofloxacin disc was placed on the agar surface, instead of punching wells. The plates were incubated for 24 hours at 37° C. The spectrum of the antibacterial effect of the ciprofloxacin disc was determined by measuring the zone of inhibition around the disc and the values were expressed in millimetres (mm)[2,12].

Statistical analysis

Statistical analysis was performed by using Graph pad Prism software (Version 8.3.1), USA through

one-way analysis of variance (ANOVA), followed by Tukey Kramer analysis as a post-hoc test.

Results and Discussion

Table 1 shows the phytochemicals present in the CME of the *parsley* leaves. The results revealed the presence of carbohydrates, steroids, and saponins. The GC-MS analysis of CME showed the presence of various bioactive compounds (Fig. 1). The chromatogram contained unique peaks demonstrating the presence of various bioactive compounds. Fig. 2 shows the structural elucidation of various bioactive compounds. The identified compounds along with their molecular formulae, molecular weights, retention times in minutes, and Probability index in percentage *include* 13-Docosenoic acid, methyl ester, (Z) ($C_{23}H_{44}O_2$, 352, 45.27, 61.38); cis-11-Eicosenoic acid, methyl ester ($C_{21}H_{40}O_2$, 324, 40.90, 34.64); 11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$, 296, 35.08, 15.10); Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$, 270, 31.15, 77.39); 15-Tetracosenoic acid, methyl ester, (Z)- ($C_{25}H_{48}O_2$, 380, 48.76, 27.75); Cyclopentanone, 3,4-bis(methylene)- (C_7H_8O, 108, 27.36, 23.83); Stigmastan-3-ol, 5-chloro-, acetate, (3á,5à)-($C_{31}H_{53}ClO_2$, 492, 54.84, 14.19); and L-Proline, 5-oxo-, methyl ester ($C_6H_9NO_3$, 143, 19.10, 16.08).

The GC-MS analysis showed eight prominent compounds, most of which were fatty acids. The fatty

Table 1 — Phytochemical analysis of the cold methanolic extract of the leaves of *Petroselinum crispum*

Phytochemicals	Observations
Carbohydrate	Present
Proteins	Absent
Alkaloids	Present
Tannins	Present
Steroids	Present
Saponins	Present
Flavonoids	Present

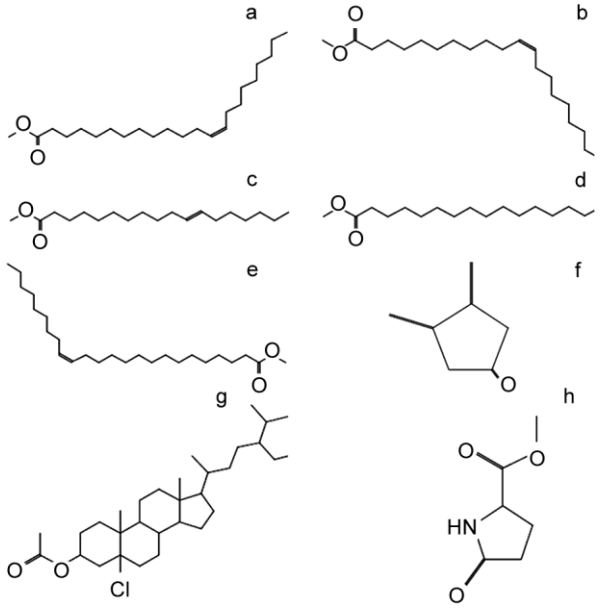

Fig. 2 — Bioactive compounds of cold methanolic extract of the leaves of *Petroselinum crispum*. a) 13-Docosenoic acid, methyl ester, (Z)-; b) cis-11-Eicosenoic acid, methyl ester; c) 11-Octadecenoic acid, methyl ester; d) Hexadecanoic acid, methyl ester; e) 15-Tetracosenoic acid, methyl ester, (Z)-; f) Cyclopentanone, 3,4-bis(methylene)-; g) Stigmastan-3-ol, 5-chloro-, acetate, (3á,5à)-; and h) L-Proline, 5-oxo-, methyl ester.

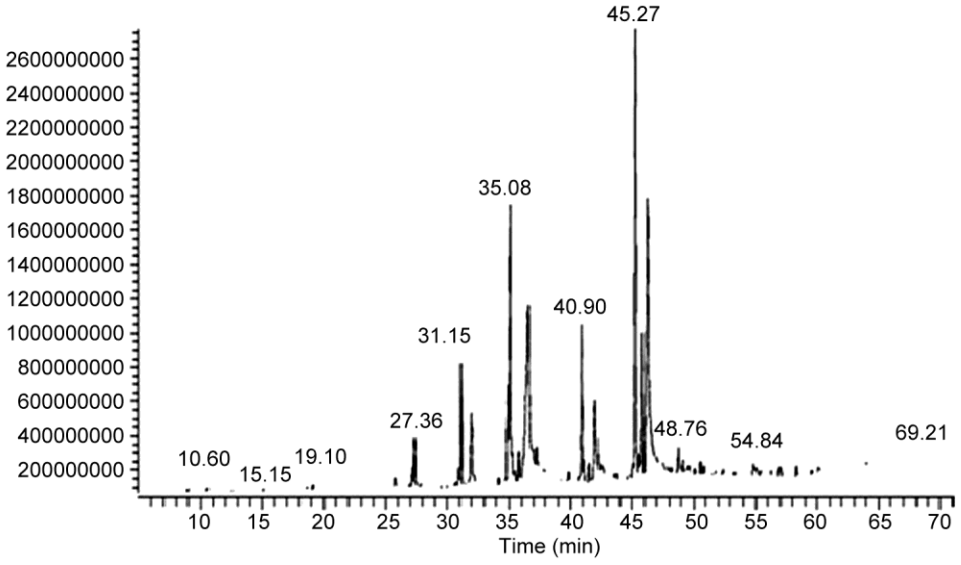

Fig. 1 — GC-MS chromatogram of cold methanolic extract of the leaves of *Petroselinum crispum*.

acid 13-docosenoic acid methyl ester (Z) elicited maximum retention time and probability index. An earlier study suggested that the fatty acid cis-13-docosenoic acid was present in high concentrations in the species of mustard seed[13]. A previous study reported the chemical composition of *Lepidium sativum* seed oil and its antibacterial and antioxidant properties[14]. In that study, 13-docosenoic acid methyl ester (Z) was identified as one of the constituents present (about 0.11%), with a retention time of 24.15 minutes. Interestingly, in this study, 13-docosenoic acid methyl ester (Z) was present to a level of 61.38 % and it eluted in 45.27 minutes.

11-Eicosenoic acid is a monounsaturated long-chain omega-9 fatty acid usually presents in plant oils. In this study, cis-11-eicosenoic acid methyl ester was identified as a major compound, followed by 13-docosenoic acid methyl ester (Z). An earlier report suggested that micro-algal fatty acids (which include 11-eicosenoic acid) showed antimicrobial activity[15]. Moreover, it has been reported that hexadecanoic acid *exhibited a good spectrum of antibacterial and antifungal efficacy*[16]. The present study also demonstrated that the major constituents of parsley leaves are 11-octadecenoic acid (stearate) methyl ester, and hexadecanoic acid (palmitate) methyl ester. An earlier report suggested that the presence of 9- octadecenoic acid methyl ester as a major constituent of Sudanese *P. crispum* demonstrated the antibacterial potential of the plant[17]. In this study, 15-tetracosenoic acid methyl ester (Z) also called methyl nervonate or nervonic acid methyl ester was identified as one of the major bioactive compounds in the CME of *P. crispum*. An earlier study showed that 15-tetracosenoic acid methyl ester was a major constituent of the seed oil of *Acer truncatum*; 15-tetracosenoic acid methyl ester, being a principal component of neural cells and neural tissue of the brain, is highly beneficial for brain health through enhancement of nerve cell function[18]. Cyclopentanone, 3,4-bis(methylene) and stigmastan-3-ol, 5-chloro- acetate were also identified in the CME of *P. crispum* in this study. An earlier study had shown the presence of stigmasta-5, 24 (28)-dien-3-ol (3á, 24Z) in *Sargassum aquifolium*[19]. Stigmasterol exerts a significant anti-osteoarthritic effect via inhibition of pro-inflammatory mediators[20].

Results from FT-IR analysis of the cold methanol extract of *P. crispum* showed the presence of unique peaks of functional groups, indicating the presence of various pharmaceutically important compounds, especially amino acids, tannins, saponins, and steroids. These were also reflected in the phytochemical tests. The functional groups present are presented in Table 2. The presence of functional groups was obvious from the FT-IR spectrum. The large parabola-shaped peak detected at 3176.08 cm^{-1} with N-H stretching indicated the presence of aliphatic amino acids. The peak at 2949 cm^{-1} indicated the presence of steroidal compounds, tannins, and saponins. The study also showed the presence of isothiocyanates and polysaccharides at 2173 cm^{-1} and 1018 cm^{-1} respectively.

The antibacterial spectrum of CME from *P. crispum* leaves is depicted in Table 3. The results indicated a narrow spectrum of antibacterial activity against the bacterial organisms screened. However, the antibacterial activity was better against Gram-negative bacteria than against Gram-positive bacteria. The antibacterial spectrum of activity of the CME of

Table 2 — Major peak values of the FT- IR spectroscopy and their respective functional groups of cold methanolic extract of the leaves of *Petroselinum crispum*

Wave number (cm^{-1})	Intensity estimation	Functional group	Nature of functional group	Possible compounds
3176	W	N-H stretching	Aliphatic amines	Amino acids
2949	S	C-H stretching	Alkane	Aliphatic compounds, steroids, Tannins, saponins, steroids
2837	S	C-H stretching	Alkane (CH_3 and CH_2)	Aliphatic compounds
2524	M	S-H stretching	Thiol	Amino acids
2173	M	-N-C=S stretching	Isothiocyanates	Isothiocyanates
2039	W	NH asymmetric stretching	Secondary amino salt	Amino acids
1652	S	C=O,C=C, N=O asymmetric stretching	Ester, Nitrate	Carboxylic acid, ester, pectin
1450	M	O-H bending	Carboxylic acid	amino acids polysaccharides
1412	M	O-H bending	Phenol or tertiary alcohol	Amino acids polysaccharides
1107	S	C-N stretching	Aliphatic amine	Aliphatic compound
1018	S	S=O stretching	Sulfonates	Starch and polysaccharides

Table 3 — A comparative antibacterial study

Organisms	Concentration CFU[#]/mL	Zone of inhibition (mm)	
		Test sample	Ciprofloxacin (5 µg/disc)
Bacillus subtilis	3×10^{-5}	5.3 ± 0.4	23.7±1.6
Staphylococcus aureus	3×10^{-4}	4.3±0.5	24.5±1.7
Streptococcus pyogenes	4×10^{-5}	4.6±1.2	26.2±1.5
Escherichia coli	3×10^{-5}	8.7±0.47	26±1.9
Pseudomonas aeruginosa	2×10^{-5}	6.3±0.5	24.3±1.2
Klebsiella pneumoniae	3×10^{-3}	5.6±1.2	24.8±1.3

Each value is the mean of n=6 batches with standard deviation by performing Tukey Kramer analysis (post hoc test). The Test sample values are significantly lesser than standard ciprofloxacin disc at $P < 0.001$, * Test sample: Cold methanolic extract of the leaves of *Petroselinum crispum*; [#]CFU: Colony forming unit.

the leaves of *P. crispum* was in the order *E.coli* (8.7±0.47 mm) > *P.aeruginosa* (6.3±0.5 mm) > *K.pneumoniae* (5.6±1.2 mm) > *B.subtilis* (5.3±0.4 mm) > *S.pyogenes* (4.6±1.2 mm) > *S.aureus* (4.3±0.5 mm). The observed antimicrobial effects might be due to the presence of cis-11-eicosenoic acid methyl ester, and hexadecanoic acid.

Conclusion

The present study has demonstrated the presence of various biomolecules in the cold methanol maceration extract of *P. crispum leaves*. The study depicted the presence of unique fatty acids such as 13-docosenoic acid methyl ester (Z), cis-11-eicosenoic acid methyl ester, stearate, palmitate, and nervonic acid methyl ester. Moreover, the extract exhibited a low spectrum of antibacterial potential. Further studies are in process to analyze the bioactive constituents using hot continuous percolation techniques, with methanol as solvent.

Conflict of interest

The authors declare that no conflict of interest is associated with this study.

References

1 Sivakumar S M, Alam M F, Safhi M M, Jabeen A, Sanobar S, *et al.*, Potency of nano-antibacterial formulation from *Sargassum binderi* against selected human pathogenic bacteria, *Braz J Pharm Sci*, 2018, **54**(4), 1-9.

2 Moni S S, Alam M F, Sultan M H, Makeen H A, Alhazmi H A, *et al.*, Spectral analysis, *in vitro* cytotoxicity and antibacterial studies of bioactive principles from the leaves of *Conocarpus lancifolius*, a common tree of Jazan, Saudi Arabia, *Braz J Biol*, 2023 , **83**, e244479.

3 Agyare C, Appiah T, Boakye Y D and Apenteng J A, Chapter 25 - *Petroselinum crispum: a Review*, Editor(s): Victor Kuete, Medicinal Spices and Vegetables from Africa, (Academic Press), 2017, 527-547.

4 Tang E L, Rajarajeswaran J, Fung S and Kanthimathi M S, *Petroselinum crispum* has antioxidant properties, protects against DNA damage and inhibits proliferation and migration of cancer cells, *J Sci Food Agric*, 2015, **95**(13), 2763–2771.

5 Mahmood S, Hussain S and Malik F, Critique of medicinal conspicuousness of Parsley (*Petroselinum crispum*): A culinary herb of Mediterranean region, *Pak J Pharm Sci*, 2014, **27**(1), 193-202.

6 Natalie Olsen R D, Why is parsley so healthy? Medical News Today, https://www.medicalnewstoday.com/articles/284490.php, 2018.

7 Linde G A, Gazim Z C, Cardoso B K, Jorge L F, Tešević V and Glamoćlija J *et al*., Antifungal and antibacterial activities of *Petroselinum crispum* essential oil, *Genet Mol Res*, 2016, **15**(3), 1-11.

8 Farzaei M H, Abbasabadi Z, Ardekani M R S, Rahimi R and Farzaei F, Parsley: A review of ethnopharmacology, phytochemistry and biological activities, *J Tradit Chin Med*, 2013, **33**(6), 815-826.

9 Gul R, Jan S U, Faridullah S, Sherani S and Jahan N, Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan, *Sci World J*, 2017, **2017**, 1-7.

10 Makeen H A, Menachery S J, Moni S S, Alqahtani S S, ur Rehman Z, *et al.*, Documentation of bioactive principles of the exudate gel (EG) from the stem of *Caralluma retrospiciens* (Ehrenb) and *in vitro* antibacterial activity – Part A, *Arab J Chem*, 2020, **13**(8), 6672-6681.

11 Sivakumar S M, Muhammad H S, Hafiz A M, Osama A M, Mohammed A B, *et al.*, Bioactive principles in exudate gel from the leaf of *Aloe fleurentiniorum*, traditionally used as folkloric medicine by local people of Aridah and Fayfa mountains, Saudi Arabia, *Arab J Chem*, 2021, **14**(11), 103400.

12 Cappuccino J G and Sherman N, *Microbiology – A Laboratory Manual*, Pearson Education Inc, USA 2014.

13 Knutsen H K, Alexander J, Barregard L, Bignami M, Bruschweiler B, *et al*., Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters, *EFSA J*, 2018, **16**(1), 1-49.

14 Alqahtani F Y, Aleanizy F S, Mahmoud A Z, Farshori N N, Alfaraj R, *et al.*, Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of *Lepidium sativum* seed oil, *Saudi J Biol Sci*, 2019, **26**(5), 1089-1092.

15 Alqahtani F, Aleanizy F S, Mahmoud A Z, Farshori N N, Alfaraj R, *et al.*, Microalgal fatty acid methyl ester a new

source of bioactive compounds with antimicrobial activity, *Asian Pac J Trop Dis*, 2014, **4**(S2), S979-S84.

16 Chandrasekaran M, Senthilkumar A and Venkatesalu V, Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L, *Eur Rev Med Pharmacol Sci*, 2011, **15**, 775-780.

17 Abdel Karim M, Sufian A, Kamal M S and Inas O, GC-MS analysis and antimicrobial activity of fixed oil from Saudi *Lepidium sativum* (crusifereae) seeds, *Int J Adv Res*, 2017, **5**(3), 1662-1670.

18 Qiao Q, Wang X, Ren H, An K, Feng Z, *et al.*, Oil content and nervonic acid content of *Acer truncatum* seeds from 14 Regions in China, *Hortic Plant J*, 2019, **5**(1), 24-30.

19 Moni S S, Alam M F, Makeen H A, Alhazmi H A, Sultan M, *et al*., Solvent extraction, spectral analysis and antibacterial activity of the bioactive crystals of *Sargassum aquifolium* (Turner) C. Agardh from Red Sea, *Nat Prod Res*, 2019, **35**(8), 1379-1383.

20 Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, *et al*, Stigmasterol: A phytosterol with potential anti-osteoarthritic properties, *Osteoarthr Cartil*, 2010, **18**(1), 106-116.