Journal of
Biomedical and Pharmaceutical Sciences

Taha and Balachandran, J Biomed Pharm Sci 2020, 3:2

Research Article Open Access

Brine Shrimp Lithality-guided Fractionation of the Medicinal Plant *Sesuvium verrucosum* Active Constituent with an Alkaloidal Structure

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Abstract

Lithality-guided fractionation of the ethanol extracts of *Sesuvium verrucosum* a halophytic plant from Bahrain, lead to the isolation of an active constituent showing a marked significant activity (LC50=21.4 µg /mL) in the brine shrimp experiment. The plant extracts have been reported to contain alkaloids in leaves, coumarins, sterols and to a lesser extent tannin. We have performed earlier a screening bioassay experiment which showed the plant, in comparison to other plants tested, to possess siginificant cytotoxicity in the crude extract. In this paper we report deconvolution steps of the active ingredient of the plant. The crude extract has been separated into four major fractions (F001-F004). The activity was shown to be residing in fraction F004 (3.5 g). A small portion of F004 was tested for the presence of alkaloids using three alkaloid testing reagents and was shown to be positive. The bulk of fraction F004 was subjected to column chromatography on silica gel (60-120 mesh) using gradient elution from hexane-CHCl3 (9:1) to CHCl3-EtOH (1:9) (10 fractions, I-X) followed by TLC (SiO_2, GF254) analysis. Only fractions VII and VIII (F005) (0.95 g) showed bioactivity (LC50=21.4 mg/mL). The two fraction were then combined and separated by preparative TLC (SiO_2, 1.5 mm layers) into 2 major bands (I & II) and only band II (72 mg) was shown to be active. The chemical structure of the solid separated from band II has been elucidated by GC-MS. GC-MS analysis shows the compound to possess a molecular ion at m/z 251 (%) indicative of a N-containg compound and a base peak at m/z 105 indicative of phenyl ketone fragment. Fragments at m/z 77 and m/z 91 are due to a phenyl ring and a tropylium ion, respectively. A tentative alkaloidal structure is suggested and was given the name: verrucosine.

Keywords: Sesuvium verrucosum; Medicinal plane; Cytotoxicity; Brine shrimp; Activity-guided isolation; GC-MS analysis' alkaloidal structure

Introduction

Sesuvium verrucosum Raf. (Aizoaceae), is a halophytic annual shrub which is related to the Sea Purslane (*S. portulacastrum*) cultivated in some countries of East Asia as a vegetable [1]. In Bahrain the shrub represents an important plant cover in the process of land reclamation [2]. The plant, locally known as Rokhama, is used in folk tradition for treatment of ear inflammations [3]. The plant extracts have been reported to contain mainly alkaloids in leaves, coumarins, sterols and to a lesser extent tannin [4]. However, there was no detailed pharmacological study on the cytotoxicity of the plant. We have, therefore, undertaken a screening bioassay experiment to test for the toxicity of the plant among other plants of similar medicinal value used in Bahrain [5]. In this experiment a statistically significant lethality to brine shrimps was observed with different concentrations of the ethanolic extracts of *S. verrucosum* in comparison to the control and other plants tested. In this paper the results of a bioactivity guided extraction, chromatographic and chemical analyses of the active ingredients responsible for the lethality of the plant, following the procedure by McLaughlin will be reported [6-8]. Apart from our earlier work on *S. verrucosum* no work to our knowledge have been reported on the bioactive constituents of the plant. A few reports have appeared recently on *S. portulacastrum* phytochemical and antibacterial properties. Ethanolic extract of *S. portulacastrum* showed antimicrobial activity against Staphylococcus aureus and *E. coli*, indicating its potential application in relation to nosocomial infections. GC-MS analysis revealed 22, 23-Dihydrostigmasterol, Gallic acid, (2R,3R)-(-)-Epicatechin and Capsaicin and considered to be the molecules responsible for the antimicrobial activity of *S. portulacastrum* [9]. Earlier fatty acids methyl esters were tested for their antibacterial and antifungal activity. Sesuvium spp is also an important source of phytoecdysteroids (insect molting hormones) and can be used as biological pesticides [10,11]. Of the various Chinese plant sources identified, Sesuvium contained the highest levels of ecdysteroids and can be used in sericulture industry to manage the silkworm rearing during the last stage of larval development [12]. In the biomedical application, 20E ecdysteriods and derivatives are used for health improvement as they have been shown to stimulate the synthesis of proteins, muscle, be adaptogenic for human immunodeficiency virus (HIV) patients and have antioxidant and tonic properties (*Sesuvium portulacastrum*, a plant for drought, salt stress, sand fixation, food and phytoremediation.) [13]. Krstenansky reviewed the Aizoace family and found to contain mesembrine alkaloids [14]. Medicinally and economically, Sesuvium containing secondary metabolites have shown a great potential as a substitute for some synthetic raw materials in the food, perfumery, cosmetic and pharmaceutical industries [15]. Sesuvium plant is used in traditional medicine as a remedy for fever, kidney disorders and scurvy [16] by the indigenous people in Africa, Latin America and in Asian countries such as India, China, Pakistan and Japan. The essential oil extracted from the leaves of Sesuvium revealed notable antibacterial activity against both gram positive and gram-negative bacteria and displayed significant antifungal and antioxidant activity [17]. The essential oil showing these activities was

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Received November 03, 2019; **Accepted** March 16, 2020; **Published** March 23, 2020

Citation: Taha A, Balachandran R (2020) Brine Shrimp Lithality-guided Fractionation of the Medicinal Plant *Sesuvium verrucosum* Active Constituent with an Alkaloidal Structure. J Biomed Pharm Sci 3: 415.

attributed to the presence of relatively higher amount of monoterpene which was actually composed of more or less content of hydrocarbon compounds such as O-cymene, 2-β-pinene, α-pinene, 1, 8-cineole, limonene, α-terpinene, α-terpinolene and camphene. The fatty acid methyl esters (FAME extract) from Sesuvium leaves have been shown to contain higher saturated fatty acids than the unsaturated fatty acids, and the extract showed antimicrobial activity against *Aspergillus fumigatus* and A. niger. Methanolic extracts of the plant contributed to its cholinesterase inhibitory activity which was comparable to the standard drug Donepezil used for treatment of Alzheimer's disease [18]. Khajuria et al. isolated a Trihydroxydimethoxyflavone 3-glucoside from *Sesuvium Portulacastrum*, (28,16a) [19], whereas Chandrasekaran et al. tested antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *S. portulacastrum* L [10]. Chou and Lu (1980) investigated [20] the growth regulation and silk production in silk worm by ecdysteroids. Kanth et al. [21] ivestigated phytoremediation and removal of salts from soil treated with tannery wastewater using *S. portulacastrum*. The same author curried studies on the use of *S. portulacastrum* for preservation of skins [22]. Whereas Martinez et al. [23] investigated Chemical composition and biological activities of essential oil from the leaves of *S.portulacastrum*.

Materials and Methods

Plant material

S. verrucosum (SV) was collected from around Isa Town Campus, University of Bahrain and Sitra Town Industrial Area in three Batches (BI, BII & BIII). Samples of the plant collection were authenticated at the Biology Department, College of Science, Isa Town, Bahrain (Herbarium classification No. 000064) The whole plant was dried in air in shade for three weeks. Plant material in each batch was chopped then pulverized using a Santos Coffee Mill and kept separately at 20°C in closed polyethylene bags. The magenta-coloured flowers in BIII were separated from the fleshy leaves and twigs and dried in a similar manner.

Extraction and isolation of different plant fractions

The powdered plants (100 g) from BI &BII were extracted continuously with 96% ethanol (1.2 L) using a set of four Soxhlet extractors (Wertheim 45/40). Whole plant extracts were obtained by complete evaporation of the solvent using a rotatory evaporator (Buchi Rotavapor Model RE 120) at 40°C. The residues left after evaporation, were combined (10.28 g), dissolved in chloroform (40 mL) followed by filtration and the filtrate was separated from droplets of water and dried (anhydrous MgSO4). Evaporation of the chloroform whole plant extract (CWPE) afforded a syrupy liquid (F001) (8.38 g), part of which was used directly in the brine shrimp (BST) experiment and was found to be active (LC50=240.4 µg/mL) (Figure 1).

Test for alkaloids

Reagents preparation

A) Mayer's reagent

Solution A: 1-36 g $HgCl_2$ in 60 ml of water

Solution B: 50 g KI in 10 ml of water

Then solution A and B transferred to a 100 ml volumetric flask and filled to the mark.

B) Dragendorff reagent

Solution A: 8.0 g Bi (NO3). 5 H_2O in 20 ml of water

Solution B: 27.2 g KI in 50 ml of water

Both solutions were mixed and allowed to stand for 24 hours, after filtration the volume is made up to 100 ml with water

Results

About 50 g of F001 were extracted with 300 ml methanol by soxhlet extractor for 8 hours. 50 mg of concentrated methanol extract was placed in a small test tube. And 1 M HCl (1 ml) was added. The mixture was stirred with a glass rod for 19 min in order to achieve complete dissolution of the alkaloids.

50 mg of F001 were used to test for the presence of alkaloids using three different alkaloid detection reagents. The three detection reagents Mayer (++), Dragendorff (+++), and Bouchardat (+++) gave positive test for presence of alkaloids from abundant (+++) to moderately abundant (++). The major part of F001 was then partitioned between a CHCl3/water (1:1) mixture (40 mL) and the chloroform part and washings (5 x 20 mL) were combined, dried (MgSO4) and concentrated to afford the chloroform soluble extract (F002) (4.6 g) (LC50=117.2 µg/mL). F002 was extracted with n-hexane to afford a hexane soluble (HE) fraction (F003) (0.5 g) (LC50=inactive) (Figure 2) and a chloroform soluble residue (SVCE-HE) (F004) (3.8 g).

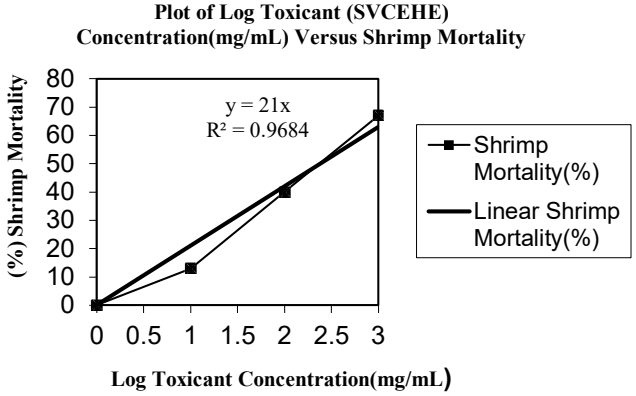

Figure 1: LC 50=240.44 ppm.

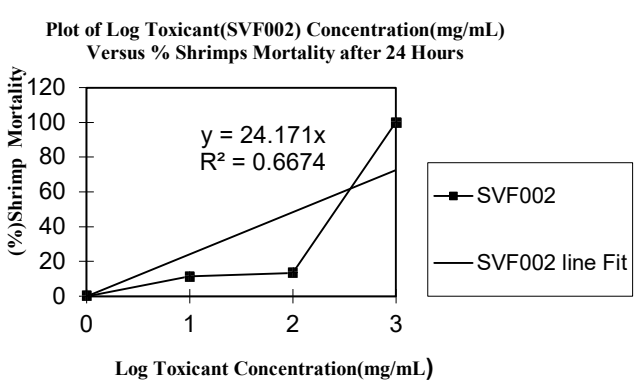

Figure 2: LC50 is 117. 5 ppm for fraction SVF002.

Flower extracts

The pulverized flowers (4.5 g) from BIII were extracted with distilled water (50 mL) for 10 minutes on a water bath, then filtered while hot. The filtrate was divided in two equal portions and one portion was boiled with 15 mL of 10% H_2SO_4 for 30 minutes, cooled (ice-water) and the precipitated flower aglycones (FAG) were collected (0.05 g) and tested for bioactivity in the BST experiment (in-active). 5 mL aliquot of the second portion were tested for the presence of free flavonoids and flavonoid glycosides (positive for both). The bulk of the second portion was then successively extracted first with CHCl3 followed by EtOAc and the glycone residues from each extraction were combined to afford the flower glycones (FG) residues (0.03 g) which were then tested in the BST experiment (inactive) (Figure 3).

Chromatographic separation and GC-MS analysis

Fraction F004 (3.5 g) was subjected to column chromatography on silica gel (100 g, 60-120 mesh) using gradient elution from hexane-$CHCl_3$ (9:1) to CHCl3-EtOH (9:1). 164 fractions were collected (Fraction Collector) and subjected to TLC (SiO2, GF254, Fluka) analysis before pooled together (fractions I-X). Only fractions VII and VIII (F005) (0.95 g) showed bioactivity (LC50=21.4 mg/mL). The two fraction were then separated by preparative TLC (SiO2, 1.5 mm layers) into 2 major bands (I & II) and only band II (72 mg) was shown to be active. The chemical structure of the solid separated from band II has been tentatively characterized by GC-MS (Figure 4).

Brine shrimp lethality test (BST)

The BST lethality experiment was performed on the different fractions according to the procedure described by McLaughlin [6,8] as follows: 20 mg of each fraction was dissolved in 2 ml of methanol and 5, 50 and 500 µL amounts were transferred to 2 dram vials (Beatson Clark Glass) to correspond to concentrations of 10, 100 and 1000 µg/ mL. Three vials were used for each concentration. The vials were dried using an air dryer to allow complete evaporation of the methanol. Control vials were prepared using 500 µL of methanol alone. Rotenone standard was prepared in a similar way but with appropriate (4x) dilution. The brine shrimp larvae, taken 48 h after initiation of hatching in natural sea water were added to each vial, and the final volume of each vial was adjusted to 5 mL using sea water. A drop of dry yeast suspension (3 mg in 5 mL seawater) was added as food to each vial. The vials were maintained under illumination After 24 h survivors were counted and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version 7 DATA TOOLKIT ANALYSIS); the LC50 values with 95% confidence intervals were determined from the best-fit line. No deaths were observed to occur in the control after 48 hours. Rotenone (Sigma) (LC50=1.23 X 10^{-2} µg/mL) is used as a positive control and methanol (500 µL) as a solvent and a negative control in the bioassay experiments [7].

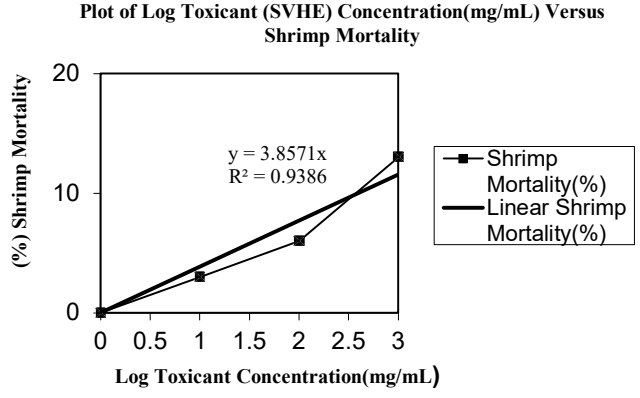

Figure 3: Hexane extract (SVHE) is inactive.

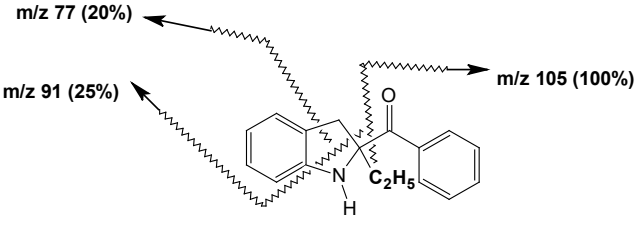

Figure 4: Fragmentation Pattern of active ingredient F005.

Brine shrimp analysis: Brine shrimp lethality test (BST) was used in a bioactivity-guided fractionation experiment to track the active ingredient in the ethanol extracts of the medicinal plant *Sesuvium verrucosum*. The result (Table 1) show that fractions VII and VIII of F004 possessed significant bioactivity (LC50=21.4 mg/mL) in comparison with values quoted in the literature [8] and the value quoted earlier for *S. verrucosum* ethanol extract [5]. This order of activity of the active ingredient in the plant is of the 1/5TH order of magnitude found for the fish poison, rotenone (LC50=4.23 mg/mL), measured under the same set of conditions of the experiment which yet gave lower activity for rotenone than that reported in the literature [7]. This could be due to slight different conditions used with the experiment from that in the literature or to the fact that the rotenone sample used in the experiment had a long shelf-life or to both. Flower extracts and hexane extracts showed no bioactivity. Table 1 shows the results for the various plant fractions tested. Table 1 gives the results of the brine shrimp after 24 hours' exposure to whole plant extracts of *Sesuvium verrucosum* (SV) and the positive control Rotenone (ROT). Rotenone, compared with negative control (methanol) which showed no mortality to brine shrimp, was highly lethal to the shrimp than the negative control. As to the different plant fractions significant variation in lethality to Artemia salina was observed with exposure to different fractions of *Sesuvium verrucosum* (SV) extracts (F001-F005). Also the degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration (10 µg/mL) to highly significant with the highest concentration (1000 µg/mL) compared to methanol. Maximum mortalities took place at concentration of 1000 µg/mL whereas least mortalities were at 10 µg/mL concentration. In other words, mortality increases gradually with the increase in concentration of SV plant extract. Similar mortality effect was found after 48 hours' exposure to each plant extract. Lethal concentrations LC50 of (SV) plant at 24 hours were obtained by a plot of percentage of the shrimps killed against logarithm of the concentrations of various fractions of the plant extract (toxicant concentration) and the best-fit line was

Fraction	Yield(%)	LC_{50} ppm
F001	8.38	240.4
F002	4.6	117.2
F003	0.5	9.2×10^{12} (inactive)
F004	3.8	-
F005	0.95	21.4
Rotenone	-	4.23

Table 1: Percentage yields and lethality (24 h) of the various fractions of *Sesuvium verrucosum* ethanol extract.

obtained from the curve data by means of regression analysis. LC50 were obtained from the best-fit line slope (Figures 1-3). This significant lethality of *Sesuvium verrucosum* plant to brine shrimp is indicative of the presence in this plant of a potent cytotoxic component which was analyzed further by using GC-MS a powerful tool for purification and identification of the active ingredient.

GC-MS analysis

Chromatographic analysis was performed on the most bioactive fraction of the plant extract using a HP 5890 GC fitted with a 5970 series mass selective detector. The chemical structure of the solid separated from band II has been elucidated by GC-MS. GC _MS was run om the Field-Desorption (FD) Mode to give the molecular ion (only m/z 1%) (Figure 5).

Discussion of the results

In order to elucidate the structure of the white solid separated by preparative TLC (mp 255-257°C) we reverted to using GC-MS analysis (Figure 6) (fragmentation pattern) which shows the compound to possess a molecular ion at m/z 251 (1%) indicative of a N-containg compound and a base peak at m/z 105 (99-100%) indicative of phenyl ketone fragment. Fragments at m/z 77 (20%) and m/z 91 (25%) are due to a phenyl ring and a tropylium ion, respectively. A tentative structure is suggested (Figure 4). Biogentically speaking the structure comes from a combination of two L-phenylalanine precursors. (L-Penylalanine is the biogenetic precursor of all Aizoaceae alkaloids). The scarcity of material prevented full characterization of the compound and only tentative structure was given with Molecular Formula C17H17NO and indole basic structure below (Figure 5) for which we suggest the name Verrucosine. Most Aizoaceae alkaloids share this basic indole structure. However, more work is needed to isolate enough material for full characterization of the active ingredient.

Results and Discussion

Brine Shrimp analysis

Because of the many advantages of the brine shrimp technique (BST) we used this technique. First is simple, secondly it is fast and reliable and thirdly after fractionation it gives you the right direction of the fraction where most of the activity lies. So it is ideal for finding out the most active fragments for follow-up. The BST technique was also found to correlate closely with the more expensive in vivo techniques. So in this case in our hand and following quide lines.brine shrimp lethality test (BST) was used in a bioactivity-guided fractionation experiment to track the active ingredient in the ethanol extracts of the medicinal plant *Sesuvium verrucosum*. The result (Table 1) show that fractions VII and VIII of F005 possessed significant bioactivity (LC50=21.4 mg/mL) in comparison with values quoted in the literature [8] and the value quoted earlier for S. verrucosum ethanol extract [5]. This order of activity of the active ingredient in the plant is of the 1/5TH order of magnitude found for the fish poison, rotenone (LC50=4.23 mg/mL), measured under the same set of conditions of the experiment which yet gave lower activity for rotenone than that reported in the literature [7]. This could be due to slight different conditions used with the experiment from that in the literature or to the fact that the rotenone sample used in the experiment had a long shelf-life or to both. Flower extracts and hexane extracts showed no bioactivity. Table 1 shows the results for the various plant fractions tested. Table 1 gives the results of the brine shrimp after 24 hours' exposure to whole plant extracts of *Sesuvium verrucosum* (SV) and the positive control Rotenone (ROT). Rotenone, compared with negative control (methanol) which showed no mortality to brine shrimp, was highly lethal to the shrimp than the negative control. As to the different plant fractions significant variation in lethality to Artemia salina was observed with exposure to different fractions of *Sesuvium verrucosum* (SV) extracts (F001-F005). Also the degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration (10 µg/mL) to highly significant with the highest concentration (1000 µg/mL) compared to methanol. Maximum mortalities took place at concentration of 1000 µg/mL whereas least mortalities were at 10 µg/mL concentration. In other words, mortality increases gradually with the increase in concentration of SV plant extract. Similar mortality effect was found after 48 hours' exposure to each plant extract. Lethal concentrations LC50 of (SV) plant at 24 hours were obtained by a plot of percentage of the shrimps killed against logarithm of the concentrations of various fractions of the plant extract (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. LC50 were obtained from the best-fit line slope (Figures 1-3). This significant lethality of *Sesuvium verrucosum* plant to brine shrimp is indicative of the presence in this plant of a potent cytotoxic component which was analyzed further by using GC-MS a powerful tool for purification and identification of the active ingredient.

GC-MS analysis

Chromatographic analysis was performed on the most bioctive fraction of the plant extract using a HP 5890 GC fitted with a 5970 series mass selective detector. The chemical structure of the solid separated from band II has been elucidated by GC-MS. GC-MS analysis (Figure 6) shows the compound to possess a molecular ion at m/z

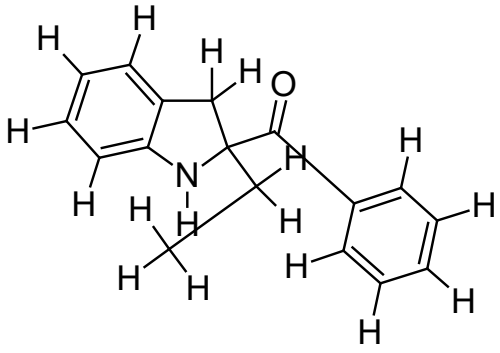

Figure 5: Proposed Structure of Verrucosine *Sesuvium verrucosum* alkaloid.

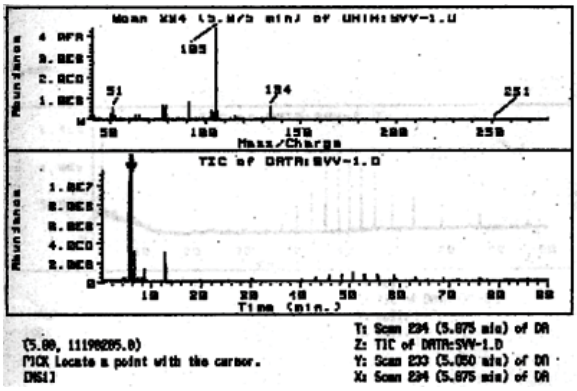

Figure 6: GC-MS TIC and fragmentation pattern of band II active ingredient.

251 (1%) indicative of a N-containing compound and a base peak at m/z 105 (99-100%) indicative of phenyl ketone fragment. Fragments at m/z 77 (20%) and m/z 91 (25%) are due to a phenyl ring and a tropylium ion, respectively. A tentative structure is suggested (Figure 4). Biogenetically speaking the structure comes from a combination of two L-phenylalanine precursors. (L-Penylalanine is the biogenetic precursor of all Aizoaceae alkaloids). The scarcity of material prevented full characterization of the compound and only tentative structure was given with Molecular Formula C17H17NO and indole basic structure below (Figure 5) for which we suggest the name Verrucosine. Most Aizoaceae alkaloids share this basic indole structure. However, more work is needed to isolate enough material for full characterization of the active ingredient.

Conclusions

The search for naturally occurring indole alkaloids was inspired by their similarity to the physiologically active alkaloids such as reserpine but in particular to the psychoactive agent such as the serotonin present in the brain.

As a result, the potential of many naturally occuring indole alkaloids as new drug leads for various psychiatric disorders is still untapped. Historically, plant-based compounds have been the source of several of the most successful drug leads or drugs used in medicine. This is indicative that more could lay in store to be discovered.

In conclusion, several indole alkaloids have been employed as antidepressants or provide lead structures for its development. Based on our findings, plants contain a reservoir of indole alkaloids which are valuable starting points for the development of future antidepressants.

Acknowledgement

The authors wish to thank Miss Fatima Al Qasimi and Sadiq Taqi for their technical assistance and the department of chemistry for funding the project.

References

1. Uphof JC (1968) Dictionary of Economic Plants, 2nd edition, Verlag Von J. Cramer, New York.
2. Phillips DC (1988). Wild Flowers of Bahrain. A Field Guide to Herbs, Shrubs and Trees, Manama, Bahrain.
3. Al-Saleh FS, Ali H and Mirza M (1993). Chemical constituents of some medicinal plants growing in Bahrain Fitoterapia, LXIV 3: 251-256.
4. Rizq AM (1986). The Phytochemistry of the Flora of Qatar, Kingprint, Richmond, UK.
5. Al Sayed H and Taha A (2000) Brine shrimp bioassay of ethanol extracts of Sesuvium verrucosum, Salsola baryosma and Zygophyllum quatarense medicinal plants from Bahrain. Phytother Res 14: 48-50.
6. McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plants constituents, Planta Medica 45: 31-34.
7. de Salzarbitoria TC, Anderson JE, Alfonso D, McLaughlin JL (1997). Bioactive furonaphthoquinones from Tabebuia barbata (Bignoniaceae) Acta Cientifica Venezolana 48: 42-46.
8. McLaughlin JL (1991). Crown gall tumors on potato discs and brine shrimp lethality: Two Simple bioassays for higher plant screening and fractionation. Methods in Plant Biochemistry 6: 1-32.
9. Al-Azzawi A, Alguboori A, Hachim MY, Najat M, Al Shaimaa A, et al. (2012) Preliminary phytochemical and antibacterial screening of Sesuvium portulacastrum in the United Arab Emirates. Pharmacognosy Res 4: 219-224.
10. Chandrasekaran M, Senthilkumar A, Venkatesalu V (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of Sesuvium portulacastrum L. Eur Rev Med Pharmacol Sci 15: 775-780.
11. Simon P, Krolman J, Koolman JA (1989). Ecdysone: from chemistry to mode of action, George Thieme-Verlag, NewYork, 254-259.
12. Nair KS, Yun-Gen M, Kumar SN (2005). Differential responses of silkworm, Bombyx mori L. to phytoecdysteroids depending on the time of administration. J Appl Sci Environ Manage 3: 81-86
13. Lokhande VH, Gor BK, Desai NS, Nikam TD, Suprasanna P (2013). A review. Agron Sustain Dev 33: 329-348.
14. Krstenansky J (2017). Mesembrine alkaloids: Review of their occurrence, chemistry, and pharmacology. Journal of Ethnopharmacology 195: 10-19.
15. Lis-Balchin M, Deans SG (1997). Bioactivity of selected plant essential oils against Listeria monocytogenes. J Appl Bacteriol 82: 759-762.
16. Rojas A, Hernandez L, Rogeho PM, Mata R (1992). Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J Ethnopharmacol 35: 127-149
17. Magawa ML, Gundidza M, Gweru N, Humphrey G (2006). Chemical composition and biological activities of essential oil from the leaves of Sesuvium portulacastrum. J Ethno-Pharmacol 103: 85-89
18. Suganthy N, Pandian SK, Devi KP (2009). Cholinesterase inhibitory effects of Rhizophora lamarckii, Avicennia officinalis, Sesuvium portulacastrum and Suaeda monica: mangroves inhabiting an Indian coastal area (Vellar Estuary). J Enz Inhibit Med Chem 3: 702-707.
19. Khajuria RK, Sun KA, Suri OP, Atal CK (1982) 3,5,4-Trihydroxy 6,7-dimethoxyflavone 3-glucoside from Sesuvium portulacastrum. Phytochemistry 21: 1179-1180.
20. Chou WS, Lu HS (1980) Growth regulation and silk production in Bombyx mori L. from phytogenous ecdysteroids. In: Hoffman JA (ed.) Progress in ecdysone research. Elsevier, Amsterdam, 281-297
21. Kanth SV, Kannan PR, Selvi AT, Saravanan P, Rao RJ, et al. (2009). Phytoremediation of soil using Sesuvium portulacastrum—part I: removal of Na+ and Cl− from tannery wastewater treated soil. J Amer Leath Chem Asso 104: 17-24.
22. Kanth SV, Keerthi PB, Selvi AT, Sarvanan P, Rao RJ, et al. (2009). Studies on the use of Sesuvium portulacastrum—part II: preservation of skins. J Amer Leath Chem Asso 104: 25-32
23. Martinez ML, Valverde T, Moreno-Magawa ML, Gundidza M, Gweru N, et al. (2006). Chemical composition and biological activities of essential oil from the leaves of Sesuvium portulacastrum. J Ethnopharmacol 103: 85-89.