Evaluation of synergistic medicinal efficacy of four alkaloidic compounds isolated from Iraqi *Allium porrum* seeds against *Staphylococcus aureus* bacteria

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Abstract: Medicinal plants are necessary benefit natural sources for chemical production of different therapies from secondary metabolism. *Allium porrum* is considered as one of sources of foods and drugs at the same time. So the current work focused on isolation and characterization of four alkaloids from seeds of this plant. These compounds are 2-piperidinone, 2-pyrrolidinone-4-methyl, adenosine -2-deoxy–N- methyl –N- (triflouro acetyl) and maymarsine by using Gas Chromatography – Mass Spectroscopy (GC-MS) technique. The medicinal activity was achieved to investigate and evaluate the synergistic action of the mixture of the four alkaloidic compounds towards *Staphylococcus aureus* pathogen. The concentrations of alkaloidic mixture were prepared with values equal to 50,100,125,150,200,250,300,375,400 and 500 mg/ml and they revealed inhibition zones with diameters represented by 31,31,32,35,35,37,41,43,43 and 45 mm. So the mixture of the four alkaloidic compounds can be used as natural therapy to treat various inflammatory caused by this pathogenic bacteria.

1. Introduction

The social and healthy need to discover natural drugs more safe than synthetic antibiotic led to development and creation in science of medicinal plants. Pre studies about the natural chemical compounds insured and proved the medical value for the chemical components abundant in the different parts of plants so the active materials were isolated, characterized and carried out successfully in treat various diseases caused by different microorganisms [1][2][3]. The medical importance of all active metabolites is absence of the side effects causing to other infections and, or inflammatory leading to a great disorder in human health. So the centers of
scientific researches established many studies about the chemical structure of active natural metabolites existing in the various of medicinal plants in order to get new drugs have no side effects, cheap, economic and available so much in the environment [4][5][6]. Antibacterial activities were achieved for various medicinal plants containing a wide range of active chemical metabolites in the different parts of these plants. Also these studies insured the great potential of active secondary metabolites for inhibition the biological and biochemical functions of living systems belonging to the pathogenic microorganisms [7][8][9]. *Allium porrum* (leek) is one of medical plant belongs to Brassicaceae family. This plant is traditionally used as a food for human in the world—wide because of its nutrition value for the human health. There are 500 genus are available in various countries but they differ in some features such as colour, maturing time and taste. Also these botanic genera are similar in chemical composition and neutral ceutical components. Different phytochemical studies were achieved about various parts of *Allium porrum* and they indicated and ensured xistence of active metabolites which included phenolics, active antioxidants sulphuric phenols and flavonoids [10][11].

Alkaloids are secondary active metabolites are found in most medicinal plants and these chemical compounds are anabolized in plants from the starting material is amino acid by different biochemical pathways by the support of required enzymes. Chemical alkaloldic metabolites are heterocyclic compounds containing nitrogen atom(s) and they have basic properties but some of them have weak acidic features whereas a few of them have neutral properties. In addition to presence of nitrogen atom(s) in the chemical structure contain sulphur, chloride, bromine and phosphorus atoms also are existing in various multi-alkaloids. Because alkaloidic metabolites are active compound so they have high medicinal efficacy to treat different diseases caused by multi-pathogens such as bacteria, virus and fungi [12][13][14]. Therefore the following research was achieved to estimate and evaluate the medicinal efficacy of synergistic interaction of mixture of four alkaloidic compounds isolated and identified from Iraqi *Allium porrum* seeds against pathogenic bacteria represented by *Staphylococcus eureus*.

2. Materials and Methods

2.1 Preparation of plant

Leek (*Allium porrum*) seeds were purchased from the local market in Al-Ashar district at Basrah Governorate in Iraq. Then the plant was taxonomied in biology department—college of education for pure sciences at University of Basrah—Iraq by specialist botanist. The seeds were washed with distilled water, dried under the shadow ground as powder with weight equal to 100 gm by electrical mill and placed in dark containers until the day of use.

2.2 Preparation of the cold aqueous extract of *Allium porrum*

The aqueous extract belonging to seeds of *Allium porrum* was prepared by mixing of 50 gm of seeds with 500 ml of distilled water as solvent in a conical flask. The contents were stirred on magnetic stirrer for 10 hours at room temperature, then the mixture was filtered by using Buchner funnel. The precipitate was removed and the filtrate was dried carefully to get a solid crude [15] with weigh equal to 6.25 gm.
2.3 Qualitative detections of alkaloidic compound

The cold aqueous extract of *Allium porrum* seeds was underwent Dragendroff reagent to detect the alkaloids existing in the plant. Fifty mg of extract was dissolved in 1 ml of distilled water in a test tube, then 2 ml of Dragendroff reagent was added into tube then orange precipitate was formed [16].

2.4 Isolation of alkaloids from *Allium porrum*

Fifty grams from seeds powder of *Allium porrum* was added into 500 ml of ethanolic acetic acid has concentration equal to (10% v/v) in conical flask and the contents were mixed and then stirred by using magnetic stirrer for 12 hours. The mixture was filtered by Buchner funnel and the precipitate was canceled then the filtrate was concentrated to a quarter of its volume. After that 5 ml of concentrated sulphuric acid was added into the filtrate and the contents were shaken well then the pH was adjusted to 9 by adding ammonia. The mixture was put in separation funnel and 20 ml of chloroform was added into mixture and contents were shaken well. Then extraction process was used and two layers were formed then organic layer volume was collected and aqueous layer was kept. This process was repeated by adding chloroform (20*3) to aqueous layer and organic layer was collected other time then the total volume was dried and the solid crude was gotten [15] with yield equal to 2.35 gm.

2.5 Gas chromatography – Mass Spectroscopy Technique

The alkaloides isolated from *Allium porrum* seeds were separated then characterized by using gas chromatography – mass spectrum procedure in GC-MS laboratory in college of agriculture in Basrah university by instrument type Shimadzu-GC-MS-QP-2101 Ultra.

2.6 Stain of pathogenic bacteria

The pathogenic bacteria isolate represented by *Staphylococcus aureus* was gotten and identified by a microbiologist in microbiology laboratory – marine science center in University of Basrah Iraq.

2.7 Preparation of culture medium

The pathogenic bacteria used in this study was treated with Muller Hinton Agar (MHA) as a culture medium for growth of this pathogen.

2.8 Estimation of Medicinal efficacy of alkaloidic compounds

To evaluate the medicinal and biochemical effect of alkaloids isolated from *Allium porrum* seeds, the method of Agar well diffusion was carried out. Two ml of Muller Hinton Agar was added into each petri dish then the medium was treated with 0.1 ml of pathogenic suspension (*Staphylococcus aureus*) has optical density equal to 0.1 with wave length is 450 nm by using spectrophotometer by using a sterilized glass spreader. Then the dishes were let for 25 minutes after that many wells were done and alkaloidic mixture was added with concentrations equal to 50, 100, 125, 150, 200, 250, 300, 375, 400 and 500 mg/ml and dishes were incubated at 37°C for 24 hours in the incubator. Finally the diameters values of inhibition zones were measured [16]

3. Results and Discussion

The health importance of *Allium porrum* plant indicates and proves the medicinal benefit resulting from existing of various active metabolites as natural drugs were used as antibacterial, antifungal, anticancer, antitumor and antiphrastic agents[17][18]. Aqueous and alkaloidic extracts were prepared with weights equal to 6.25 and 2.35
respectively therefore the extraction percentages were 25 and 9.4% as shown in table 1. This insures that the percentages were excellent and this chemical case proves abundance of different active chemical compounds in the both extracts.

**Table 1.** Extraction percentages of cold aqueous and alkaloidic extracts of *Allium porrum* seeds

| No. | Extract Sort   | Plant powder weight (gm) | Extract weight (gm) | Extraction percentages (%) |
|-----|----------------|--------------------------|---------------------|----------------------------|
| 1   | Cold aqueous   | 25                       | 6.25                | 25                         |
| 2   | Alkaloidic     | 25                       | 2.35                | 9.4                        |

It is noticed that the extraction percentage of cold aqueous extract is higher than the alkloidic extract so this biochemical state illustrates presence of more active metabolitic compounds in the aqueous extract than alkaloids.

Table 2. shows the primary qualitative analysis of cold aqueous extract of *Allium porrum* seeds by using various chemical reagents depending on the chemical nature of secondary metabolites.

**Table 2.** Primary detection on active metabolites in cold aqueous extract of *Allium porrum* seeds

| Reagent sort         | Test result | Test Indication                  | Conclusions          |
|----------------------|-------------|----------------------------------|----------------------|
| Molisch              | +           | Violet ring                      | Existence of carbohydrates |
| Benedict             | +           | Red precipitate                  | Existence of glycosides |
| Dragendroff          | +           | Orange precipitate               | Existence of alkaloids |
| FeCl₃(1%)            | −           | No bluish-green colour           | Absence of phenols   |
| Lead acetate(1%)     | −           | No white-light brown precipitate | Absence of tannins   |
| Ethanolic KOH (5N)   | −           | No yellow precipitate            | Absence of flavonoids |
| Liberman-Burchard    | +           | Deep blue–greenish colour        | Existence of steroids |
| Ninhydrin(1%)        | −           | No violet colour                 | Absence of amino acids |

From table 1, it was noticed that the cold aqueous extract belonging to *Allium porrum* contains carbohydrates, alkaloids, glycosides and steroids but phenolics, tannins and amino acids are absent. Many researchers carried out different experiment and analysis about phytochemical content of various parts of *Allium porrum* including seeds and they found different chemical metabolitic classes such as phenolic compounds, alkaloids, flavonoids and glycosides [19][20]. Alkaloids as secondary active materials were isolated by using different polar solvents and these potent compounds were gotten from the organic layer and this process insures existence of non-polar alkaloid compounds. Many researches were established for isolation, separation and characterization of plant alkaloids in a high purity [21][22].

Gas chromatography–mass spectroscopy technique was applied for chemical identification of alkaloidic compounds abundant in the alkaloidal extract were chemically separated by gas chromatography (GC) and four peaks were appeared in alkaloidal extract. At the beginning the active alkaloids with different retention times.
then these active metabolites were inserted to mass spectrophotometer instrument and each alkaloidic compound was indicated with mass spectrum has various peaks depending on structural groups and relation between mass and charge. The mass spectra insured that the active alkaloidic compounds are 2-piperidinone, 2-pyrrolidnone , 4-methyl,2-deoxy –N-methyl -(n-(tri-fluoroacetyl) and maymyrsine as shown in figures 1 , 2 , 3 and 4.

Figure 1. Mass spectrum of 2-piperidinone

Figure 2. Mass spectrum of 2-pyrrolidnone-4-methyl

Figure 3. Mass spectrum of adenosine 2-deoxy -N- methyl –N- ( tri fluoro-acetyl )

Figure 4. Mass spectrum of maymyrsine

Because the medicinal efficacy and biochemical potential of alkaloids therefore the synergistic interaction of the four alkaloidic compounds from *Allium porrum* seeds was achieved and studied to investigate, estimate and evaluate the medical effect of the mixture of these alkaloids against growth of *Staphylococcus aureus* pathogen. Table (3) represents the biochemical role and medicinal activity belonging to multi- concentrations were prepared from mixture of four alkaloidic compounds of seeds of *Allium porrum*.
Table 3. Medicinal efficacy of synergistic interaction of alkaloidic mixture isolated from *Allium porrum* seeds.

| Pathogenic bacteria Kind | Alkaloidic compounds mixture | Concentration (mg/ml) | Inhibition zone diameter (mm) |
|--------------------------|------------------------------|-----------------------|-------------------------------|
| *Staphylococcus aureus*   | 2-piperidinone               | 50                    | 31                            |
|                          | + 2-pyrrolidinone-4-methyl   | 100                   | 31                            |
|                          | + adenosine2-deoxy-N-methyl-N-(tri-flouroacetyl) | 125 | 32 |
|                          | + Maymyrsine                 | 150                   | 35                            |
|                          |                              | 200                   | 35                            |
|                          |                              | 250                   | 37                            |
|                          |                              | 300                   | 41                            |
|                          |                              | 375                   | 43                            |
|                          |                              | 400                   | 43                            |
|                          |                              | 500                   | 45                            |

It is noticed from table 3., the increase of concentration of alkaloidic mixture showed an increasing in diameters values of inhibition zone where the concentrations (50, 100, 125, 150, 200, 250, 300, 375, 400, and 500 mg/ml) recoded inhibition diameters equal to 31, 31.32, 35, 37, 41, 43, 43, and 45 mm. The biochemical explanation of this status belongs to high antibacterial activity of alkaloids mixture with increase of concentration leading to inhibit or kill most *Staphylococcus aureus* bacteria [23]. plant alkaloidic metabolites have chemical ability for bonding with nucleic acids are DNA and RNA existing in the living cell of pathogenic bacteria leading to destruct or inhibit the chemical and biological roles of these acids. Also multi studies insured and proved chemical capability of natural alkaloids to inhibit the metabolism of amino acids, lipids, carbohydrates and proteins through the hydrogen bonding between nitrogen atom present in alkaloids with hydrogens of these biochemical compounds [24][25][26].

Also the synergistic interaction of natural alkaloidic compound is chemically very powerful against the action of multi-enzymes abundant in the living cell of pathogens including *Staphylococcus aureus* by hydrogen bonding with thiol (-SH) group present in these enzymes leading to break the sulphuric bridges then denaturation of chemical nature belonging to these enzymes[27][28].

4. Conclusions

The synergistic interaction of the natural alkaloidic mixture containing four active chemical compounds represented by 2-piperidinone, 2-pyrrolidinone-4-methyl, adenosine-2-deoxy-N-methyl –N-(triflouro acetyl) and maymyrsine isolated from Iraqi *Allium porrum* seeds showed the high chemical ability and medicinal efficacy for inhibition of biological and physiological activities of *Staphylococcus aureus* pathogen causing multi-diseases. Also it was proved that the increase of concentration of alkaloids gave more inhibition diameters values leading to kill most of the colonies of this bacteria. So the natural mixture of alkaloidic compounds of leek can be carried out as plant therapy against this pathogenic micro-organism and comparison of the medicinal efficacy of alkaloids characterized from this plant with antibiotics which target *Staphylococcus aureus* bacteria.
5. References

[1] Ghonson, S.S. 2015. Chemical Detection of some active compounds in Egg plant (solanum melongena) callus as compared with fruit and root content . Int. J. Curr. Microbiol. App. Sci, 4(5):169-165.

[2] Mohaddese, M. and Atefeh, M. 2014. Antimicrobial activity of Capparis spinosa as its usages in traditional medicine, Herba Polonica, 60(1):1-9.

[3] Nazia, M.A.C. and Perween, T. 2008. In vitro antibacterial activities of kalonji, cumin and poppy seeds pak. J. Bot, 40(1):461-467.

[4] Padmini, E., Valarmathi, A. and Rani, M.U. 2010. Comparative analysis of chemical composition and antibacterial activities of Mentha spicata and Camellia sinensis. Asian J. Exp. Biol. Sci, 4:772-781.

[5] Al-Maliki, A.D.M; Abd, Al-Majeed, M.I; Al-Abdal, M.A.J. and Esmaeel, B.A. 2017. Characterization, estimation and evaluation of antifungal activity of lipids isolated from Iraq capparis spinosa leaves buds, J. Med. sci. and clin. Res, 5(11):2979-29808.

[6] Jeeva, S., Johnson, M. and U; Afridi, H.H; Khan, M.A.; Saleem, U.; Najeeb, U.; Hussain, J.; and Khan, M.A. 2011. Composition of phytochemical constituents and antimicrobial activities of Mentha spicata from four northern districts of Khyber. Pakh. turkhwa. J. appl. pharm. Sci, 1(7):72-76.

[8] Mahmoud, I.N.; El-Sayed, A.A. and Amany, A.S. 2010. Secondary metabolites and bioactivities of Myrurus communinis. Phatamaco. Res, 26(6):325-329.

[9] Al-Maliki, A.D.M and Bader, S.Q. 2012. Isolation and identification of tannins from Cuminum cyminum L. seeds and study of their medicinal activity against selective types of pathogenic fungi J. Missan Res, 8(16):147-161.

[10] Irkin, R.; Koru kuoglu, M. 2007. Control of Aspergillus niger with Garlic, Onion and Leek Extracts. African J. Biotechn., 8(4):384-387.

[11] Hero, F.S.A. and Jwan, D.T. 2012. Antibacterial activity of Leptium sativum and Allium porrum extracts and Juices against some Gram positive and Gram negative bacteria. Med. J. Islamic world Acad. sci, 20(1):10-16.

[12] Kabera, J.N.; Semana, E., Musa, A.R. and He, X. 2012. Plant secondary metabolites, biosynthesis, classification, function and pharmacological properties. J. pharm and pharmaceut., 2:377-392.

[13] McBrien, N.A.; Stell, W.K. ands Carr, B. 2013. How does atropine exert its anti-myopia effects, opthalmic and physio optics., 33(3):373-378.

[14] Simera, M.; Pollacek, J. and Tak us, G. 2010. Central antitissusive effect of codeine in the anesthetized rabbits. Europ. J. Med. Res., 115:184-188.

[15] Harborne, J.B. 1984. Phytochemical methods, 2 nd.ed., Chapman and Hall London, Ulk.

[16] Cowan, M.M. 1999. Plant products as antimicrobial agents. J. Clin. Microb. Rev., 124:564-582.

[17] Mehrabian, S. and Larry-Yazdy, H. 1992. Antimicrobial activity of Allium sativum, Allium cepa and Allium porrum (Liliaceae) against enteric pathogens (Enterobacteriaceae), Int. Soc. For Horticult. Sci, 319(4):177-182.

[18] Cvetnic, Z.; Pepeljnjak, S.; Stjepan, V. 2003. Knezevic of leek extract, Allium porrum L. (Liliaceae) Microb. soc., 7:4-7.

[19] Packia, L.N.C., Viveka, S.J.; Jeeva, S. and Raja, B.J. 2015. phytochemicals in Allium species and its analytical Methods- A review. Int. J. Inst. Pharm. And life sci., 5(1):1-22.

[20] Ernesto, F.; Virginia, L.; Orazio, T.S. and Carla, C. 2001. The flavonoids of leek, Allium porrum J. phytochemistry, 57(4):565-569.

[21] Dima, M.; Anne-Sylvie, F.; Emmanuel, P.; Tayssir, H.; Naneg, N.; Christine, F.; Xavier, F. and Farid, C. 2014. Chemical composition, antibacterial and antioxidant activities of six
essentials oils from Alliaceae family. *Molecules*, 19:20034-20053.

[22] Al-Saadi, J.H.; Al-Maliki, A.D.M. and Aouda, M.A. 2016. Medicinal activity of alkoldic compound 1-H-pyrido(2,3-b)indole isolated from Solanum melongena cortex against some pathogenic bacteria. *J. Nat. Sci Res.*, 6(20):80-85.

[23] Wisseni, A.W. and Brahim M. 2016. Charactenzation of myrle seeds (Myrtus communin var. bacteria) as a source of lipids,phenolic and antioxidat activities. *J. food and drug analysis*, 24(2):316-322.

[24] Mohammad, S.R.; Mohammad, Z.R.; Md, A.; Rasheduzzaman, C. and Mohammad, A.R. 2019. Antimicrobial activity of some indigenous plant of Bangladesh. *Res.J.Biotech*, 14(6):88-95

[25] Mona, Gh.; Sina, O. and Mohammad, B.O. 2016. Review of anti-inflammatorhy herbal medicines. *Adv. In pharmacol. Sci*, 11:1-110.

[26] Packia, L.; Vevika, S.; Madepalli, B.V. and paja, B.J. 2015. Phytochemical screening and in vitro antibacterial activity of Allium sativum extract against bacterial pathogen. *J.Sci.*, 5(5):281-285.

[27] Hamomoouchi, M.; Elarak, K.; Tantoui, N.E. and Agoumi, A. 2004. Eluicidation of antibacterial and antifungal properties of essential oils of Eucalyptus. *Plants Med. Ethphyto.*, 24:278-289.

[28] Leen, O., Ahmad, S. and Roula, M.A. 2019. Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. *Front. Microbiol.*, 10(911):1-28.