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Research Article

Antibacterial Activity of *Asphodelin lutea* and *Asphodelus microcarpus* Against Methicillin Resistant *Staphylococcus aureus* Isolates

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ABSTRACT

Objective: the present study aimed at evaluation of antibacterial activity of *wild local Asphodelus microcarpus* and *Asphodeline lutea* against methicillin resistant Staphylococcus *aureus* (MRSA) isolates.. Methods: Antimicrobial activity of the crude extracts was evaluated against MRSA clinical isolates using agar wells diffusion. Determination of minimum inhibitory concentration(MIC)of methanolic extract of two studied plants was also performed using tetrazolium microplate assay. Results: Our results showed that different extracts (20 mg/ml) of aerial parts and bulbs of the studied plants were exhibited good growth inhibitory effect against methicilline resistant *S. aureus* isolates and reference strain. The inhibition zone diameters of *A. microcarpus* and *A. lutea* ranged from 9.3 to 18.6 mm and from 6.6 to 15.3mm respectively. All extracts have better antibacterial effect than tested antibiotics against MRSA isolate. The MIC of the methanolic extracts of *A. lutea* and *A. microcarpus* for MRSA fell in the range of 0.625 to 2.5 mg/ml and of 1.25-5 mg/ml, respectively. conclusion:The extracts of *A. lutea* and *A. microcarpus* could be a possible source to obtain new antibacterial to treat infections caused by MRSA isolates. Further studies on isolation of phyto-constituents and both in vitro and in vivo evaluation of pharmacological activities of isolated bioactive constituents of the crude extracts are recommended as future works.

Keywords: Anibacterial, *Asphodelus microcarpus*, *Asphodeline lutea*, methicillin resistant, *S.aureus*, MIC.

INTRODUCTION

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. According to the World Health Organization, infectious diseases accounting for approximately 50% of all deaths in tropical countries[1]. One of the more alarming trends in clinical microbiology has been the increasing incidence of resistance to antimicrobial agents among pathogens causing nosocomial as well as community-acquired infections[2]. Among the more important emerging resistance problem is methicillin resistant Staphylococci, which has gained much attention in the last decades, because it has become a major hospital-acquired pathogens that are very difficult to cure as these strains are resistant against almost all clinically available antibiotics[3,4]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds and offer considerable potential for the development of new agents effective against infections currently difficult to treat[5,6]. The primary benefit of plant derived medicines is due to their availability, fewer side effects and reduced toxicity[7,8].

Asphodelus microcarpus Salzm. et Vivi (Asphodelaceae) is a stout robust herb with roots of several spindle-shaped tubers, widely distributed over the coastal Mediterranean region[9]. It has been reported in the ethnobotanical literature for use as a diuretic, for otitis and toothache in Algeria[10], a wild food source in the Medieval Levant[11], and a skin emollient, lenitive and treatment for lung diseases in Sardinia[12]. Antioxidant and anticancer propertied were also reported[13]. Flower of *Asphodelus microcarpus* used as Emollient, lenitive and for lung diseases[14].

The genus *Asphodeline* belongs to the Asphodelaceae family (until recently included in the family Liliaceae) and comprises of 14 species worldwide. It has fleshy roots and fragrant, starry flowers that are yellow in May to June. It grows up to 4 ft in well-drained soil. Its foliage is blue-green and grassy, with tall, narrow flower spikes[15]. Several *Asphodeline* species are consumed in salads, others have significant applications in traditional medicine[16]. The leaves of *Asphodeline* represent a good source of proteins of good nutritional value in addition to functional compounds, such as polyphenols and dietary fibre[17].

Asphodeline lutea (L.) Rchb. is a wild plant traditionally as food. The ancient Greeks roasted the roots like potatoes and ate them with salt and oil or mashed them with figs. In folkloric medicine, it used as an antispasmodic and diuretic[19]. Antioxidant properties of that plant were also reported, but its benefits of other medicinal properties are not well studied[19].

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Aim of study

The medicinal plants would be the best source for obtaining a variety of drugs. These evidences contribute to support and quantify the importance of screening natural products. The antibacterial activity of *Asphodelus microcarpus* and *Asphodeline lutea* studies are rare. In addition, the antimicrobial activities of plant extract against tested bacteria differed, according to location[20,21]. Therefore, the present study aimed at evaluation of antibacterial activity of *wild local Asphodelus microcarpus* and *Asphodeline lutea* against methicillin resistant Staphylococcus *aureus* (MRSA) isolates.

MATERIAL AND METHODS

Plant Samples

The plants were collected collected in June 2014 near the town of Maart Al- Numan. Identification of plants was confirmed by Dr. Ahmad Jaddouh in the faculty of Agriculture Engineering, Aleppo University, Syria. Aerial parts and bulbs were separated and dried at room temperature with good ventilation under shade. The dried parts were powdered using a mechanical grinder.

Extraction Procedures

The powdered plants were separately extracted by ultrasonic assisted extraction at 40 kHz with five folds of water, methanol or ethanol at 40ºC for 1 h. The extract was filtered through Whatman No.1 papers and the residue was re-extracted twice with the same volume solvent. The combined extracts were evaporated at 40 °C (rotary evaporator Büchi R-215, Flawil, Switzerland) to remove solvent. The obtained extract was evaporated under reduced pressure to dryness. The dried extracts were kept in refrigerator at 4˚ C until being used. Working extracts were performed by dissolving in 5% (v/v) to make 20 mg/ml.

Bacterial isolates

The MRSA clinical isolates used in this study were from patients presenting with symptoms of *S. aureus* associated diseases. The isolates were identified as *S. aureus* according to colonial and microscopic morphology, positive catalysis, hemolysis on blood agar, coagulase production and growth on mannitol salt agar. An oxacillin (1 µg) disk (Hi-media) was used for detection of Methicillin resistance as described by Datta *et al.* 2011[22]. Isolates with zone sizes 10 mm were considered methicillin resistant. A reference strain (*S. aureus* ATCC 25923) was also included.

Antibacterial testing

Agar diffusion test

Susceptibility of tested isolates to antibiotics, was determined using disk diffusion test according to (NCCLS)[23]. Antibacterial agents from different classes of antibiotics were used which included penicillin, oxacillin, augumentin, cefuroxime, ceftriaxone, cefpodoxime, gentamycin, norofloxacin,levofloxacin, azithromycin, vancomycin (Himedia Labs, India).

The antibacterial activity of the plant extracts was investigated using agar well diffusion method. The bacterial isolates was first enriched in nutrient broth for 18h before use. Growth turbidity was adjusted to a of 0.5 MacFarland standards containing approximately 10^8 (CFU)/ml. 90-mm diameter petri plates containing 25 ml Mueller-Hinton Agar (Hi-media) was seeded with 0.1ml of suspension of the test isolate. Seeded plates were allowed to stand for a while at room temperature before wells were bored using a cork borer (6mm diameter). Wells were filled with 100µ of each extract. dimethyl sulfoxide (DMSO) 5% (v/v) was used as negative control. The dishes were preincubated at 4°C for 2 h to allow uniform diffusion into the agar. After preincubation, the plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed. Strains with inhibition zones were considered sensitive to the extract, those without such a zone were considered resistant[24].

Minimum inhibitory concentration (MIC) assay:

The (MIC) of test microorganisms was determined by using tetrazolium microplate assay described previously with some modification. This assay was performed using flat bottom 96-well clear microtitre plates. 50µl of bacterial suspension of respected organisms were added to the wells and 50µl working solution of plant extracts was serially diluted (50mg/ml -3.125). One column H was kept as drug free control and one row was kept as a media control (without organisms). The culture plate was sealed with parafilm and kept in incubator at 37ºC for 24 hour. The MIC of samples was detected following addition (10µl) of 0.2mg/ml of p- iodonitrotetrazolium chloride in all the wells and incubated at 37°C for 30 min. Microbial growth was determined by observing the change of colour p-iodonitrotetrazolium chloride (INT) in the microplate wells (pinkish-red formazan when there is growth and clear solution when there is no growth). MIC was defined as the lowest sample concentration showing no colour change (clear) and exhibited complete inhibition of bacterial growth[25].

Statistical analysis

All assays were performed in triplicate in three independent experiments. Results were expressed as means ± S.D. All statistical analysis, including ANOVA was done in SPSS Version 17.0. Differences were considered significant when $p< 0.05$.

RESULTS AND DISCUSSION

The antibiotic susceptibility pattern of the clinical isolates and reference strain was shown in Table 1. According to NCCLS, reference strain *S.aureus* 25923 was sensitive to all tested antibiotics except penicillin. Whereas, All studied clinical isolates were resistant to all tested antibiotics belonging to different classes beside resistant to methicillin that demonstrated using oxacillin disk diffusion test. This result indicated the multi-drug resistance phenotype of the isolates and Inefficiency of these antibiotics as therapeutic agents to treat diseases caused by these isolates. MRSA bacteria have become a major health risk, in terms of both nosocomial and community-acquired infections[4]. The demand for new effective antimicrobials is urgent and of great importance in the clinical health to combat such clinical isolates.

Table 1: Resistance profile of MRSA Isolates

MRSA isolates	S1	S2	S3	S4	S5	ATCC25923
Penicillin 10U	-	-	-	-	-	24.3±0.6
Oxacillin 1µg	-	-	-	-	-	18.6±0.6
Augumentin 20/10 µg	10±1	9.6±0.6	11.3±0.6	11±1	10.6±0.5	26±1
Cefuroxime 30 µg	7.6±0.3	9.6±1.1	7±1	9.3±0.3	10.3±0.6	27.5±0.5
Ceftriaxone 30 µg	8.3±1.1	8.3±1.1	7.5±0.5	9±1	9.6±0.6	27.6±0.6
Cefpodoxime 10 µg	10±1	11±1	11.6±0.3	10.3±0.6	11.6±0.3	31±1
Gentamycin 10 µg	7±1	9.3±1.5	8±1	8.3±0.6	9.6±0.6	18±1
Azithromycin 15 µg	11.3±0.6	9.3±0.6	10.3±0.6	11.3±0.6	11±1	22.3±0.6
Norofloxacin 10 µg	10.3±0.6	10.3±1.1	12.3±1.5	11±1	14±1	24.3±1.1
Levofloxacin 5 µg	11±1	10.6±0.6	10.3±0.6	12.3±0.6	12.3±1.1	25±1
Vancomycin 30 µg	8±1	9.3±1.1	8±1	9.3±0.6	8.6±0.6	20.6±0.6

Table 2: Mean inhibition zones diameter ± SD (mm) induced by different extracts of *Asphodeline lutea*

MRSA isolates	Water extract		Methanol extract		Petroleum ether extract	
	Aerial parts	Bulb	Aerial parts	Bulb	Aerial parts	Bulb
1	10± 1	12 ±1	15± 1	21.3± 1.5	15.3±0.6	18 ±1
2	9.3±0.6	11.6±0.3	15.3±0.6	19.3± 0.6	15.6±0.6	18.6±0.3
3	10.6±0.6	14.6±0.6	14.6±0.6	19.6± 0.6	16± 1	17.6±0.6
4	9.6±0,6	9.3±0.6	15.6±0,6	19.3± 0.6	17.3±0.6	17.3±0.6
5	9.6±0.6	11±1	13.6±0.6	18.3± 1.5	15.6±0.3	17±1
ATCC	11.6±0.6	12.6±0.6	13.3±0.6	16.3± 1.5	15±1	16.6±0.6

Allied with this demand is the need for assays to detect new and previously undiscovered antimicrobials from plant sources. The MIC of the methanolic extracts of *A. lutea* and *A. microcarpus* for MRSA fell in the range of 0.625 to 2.5 mg/ml and of 1.25-5 mg/ml, respectively Table 4. In general, all extracts have a significantly better antibacterial effect than tested antibiotics against MRSA isolate ($P \leq 0.05$). In addition, the MRAS isolates were more sensitive to plant extracts than *S.aureus* reference strain but no significant difference was found ($P \geq 0.05$)

The current results are in agreement with different previous studies reported the antibacterial effect of *Asphodeline lutea* and *A. microcarpus*[26,27,28,29]. However, Abuhamdah *et al*. (2013) found that *A. microcarpus* ethanolic extract was completely inactive against all tested bacteria[30]. In the current study, water extract has less activity than methanol and petroleum ether extracts. Shtayeh *et al*. (1998) proved the antibacterial effect of *A.lutea* growing in palastine and they noticed better activity of ethanolic extract against *S.aureus* and *C.albicans* comparing with water extract[18]. Eloff (1998) examined a variety of extractants for their ability to solubilize antimicrobials from plants and ranked them in the order methanol, ethanol, and water[31]. This might be due the lack of solubility of the active constituents in aqueous solutions. Most antimicrobial active components that have been identified are not water soluble and thus organic solvent extracts have been found to be more potent[32,33]. Our results showed that activity *Asphodeline lutea* methanol extract of aerial parts was superior to the activity of the bulbs activity against studied isolates. Whereas, the activity of petroleum ether extract of the bulbs was better than aerial parts activity. On the other hand, methanol extract *A. microcarpus* bulbs was more active than aerial parts and petroleum ether extract of aerial parts showed best activity among all extracts of this plant. Different parts of the same plant may synthesize and accumulate different compounds or different amounts expression, which in turn, affects antibacterial activities and other biological properties of the plant extracts[34]. Many studies have confirmed that the amount and composition of phenolic and flavonoid compounds is diversified at the sub-cellular level and within plant tissues as well[35].

The antibacterial activity of *A. lutea* and *A. microcarpus* demonstrated in the current Study indicates the presence of active compound that mentioned in previous research. The secondary metabolites belonging to the different classes have been reported and *A.microcarpus* and *A. lutea* which proved to have antibacterial effects[36,37]. Lipids, carbohydrates, sterols, triterpenes, anthraquinones and arylcoumarins have been isolated from *A. microcarpus*[26]. On the other hand, anthraquinones, flavonoids and benzene/naphthalenes have been reported in *A. lutea.* The anthraquinones including chrysophanol, aloe-emodin, physcion rhein**,** asphodeline **.** Kaempferol, luteolin and guercetin as flanonoids was also found[38]. Ghoneim *et al*.

Table 3: Mean inhibition zones diameter ± SD (mm) induced by different extracts of *Asphodelus microcarpus*

MRSA isolates	Water extract		Methanol extract		Petroleum ether extract	
	Aerial parts	Bulbs	Aerial parts	Bulbs	Aerial parts	Bulbs
1	7.6± 0.6	9.6± 0.6	10.3±0.6	11.3±0.6	16.6 ±0.6	10.3±0.6
2	-	7.6± 0.3	9.3 ±0.6	11.3±0.6	14.3±0.6	9.3 ±0.6
3	7± 1	7± 1	8.6±0.3	11.6±0.6	13.6±0.6	10.6±0.3
4	6.6± 0.6	6.6± 0.6	10±1	12 ± 1	13 ± 1	11±1
5	-	7±1	7.6±0.6	9.3±0.6	13±0.6	9.6±0.6
ATCC	7.3± 0.6	7.3± 0.6	10.6± 0.3	10.3±0.6	15.3±0.6	12.6± 0.3

Table 4: MIC values *of* methanolic extract of two plants (mg/ml)

MRSA isolates	*Asphodeline lutea*		*Asphodelus microcarpus*	
	Aerial parts	Bulbs	Aerial parts	Bulbs
1	1.25	0.625	1.25	2.5
2	1.25	0.625	2.5	1.25
3	1.25	0.625	2.5	1.25
4	1.25	0.625	2.5	1.25
5	2.5	0.625	5	1.25
ATCC	2.5	1.25	5	2.5

(2013) isolated several active compounds from ethanol extract of *A. microcarpus* with different biological activity in which ramosin, emodin , and aestivin have shown to have antibacterial effect against MSRA[9]. In addition, Ghoneim *et al.* (2014) isolated five compounds identified as asphodosides A-E (1–5) by fractionation of the ethanolic extract of *A. microcarpus*, asphodoside B and D have good activity against MRSA[27]. Moreover, Uysal *et al*. (2016) manifested the *A. lutea* activity against ten isolates of MRSA by determination of MIC that was 6.25 to mg/ ml for all isolates[29].

Anthraquinones are considered important chemotaxonomic markers for plants in the family Asphodelaceae and species of that family have been shown to possess 1,8-dihydroxyanthraquinones[38]. The Weia *et al*. (2014) suggested that the antibacterial activity of 1,8-dihydroxy-anthraquinone is due to its interaction with the cell wall and cell membrane, by which it increases the permeability of the cell envelope and leads to the leakage of cytoplasm and the deconstruction of cell[39].

From this study, the plant extracts were found to have antibacterial activity against MRSA clinical isolates. However, to explain the mode of action, the active phytocompounds of these plants used against multidrug-resistant bacteria and their toxicity have to be determined by additional studies.

CONCLUSION

The extracts of *A. lutea* and *A. microcarpus* could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant isolates. However, it is necessary to isolate the active constituents and determine their toxicity, side effects and pharmaco-kinetic properties.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENES

1. WHO. World Health Report, World Health Organization, Geneva, Switzerland, WHO Publications Office 2003;1-50.
2. Spellberg B, Bartlett JG, Gilbert DN. The future of antibiotics and resistance. New England Journal of Medicine 2013; 368: 299–302.
3. Boucher HW, Talbot GH, Bradley JE, Edwards Jr, Gilbert D, Rice LB. Bad bugs, no drugs: no escape! An update from the infectious diseases society of America. Clinical Infectious Disease 2009; 48:1–12.
4. Abdulgader SM, Shittu AO, Nicol MP, Kaba M. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Africa: a systematic review. Frontiers in Microbiology 2015;6:348.
5. Sibanda T and Okoh AI. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. African Journal of Biotechnology 2007; 6 (25): 2886-2896.
6. Mahady GB. Medicinal plants for the prevention and treatment of bacterial infections Current Pharmaceutical Design 2015; 11: 2405-2427.
7. Tiwari S. Plants: A Rich Source of Herbal Medicine. Journal of Natural Products 2008; 1:27-35.
8. Jinukuti MG. and Giri A. Antimicrobial activity of phytopharmaceuticals for prevention and cure of diseases. Annals of Phytomedicine 2013; 2 (2): 28-46.
9. Ghoneima MM., Maa G, El-Helad AA, Mohammadd AI, Kottobd S, El-Ghalyd S, Cutlerc SJ, Rossa SA. Biologically Active Secondary Metabolites from *Asphodelus microcarpus*. Natural Product Communications 2013; 8(8):1117-1119.
10. Sarri, M., Mouyet, F.Z., Benziane, M., and Cheriet, A Traditional use of medicinal plants in a city at steppic character (M'sila, Algeria). Journal of Pharmacy & Pharmacognosy Research 2014; 2, 31-35.
11. Rubin, R. The Melagria: On Anchorites and Edible Roots in Judaean Desert. Liber Annuus 2002; 52, 347-352.
12. Loi, M.C., Maxia, L., and Maxia, A. Ethnobotanical comparison between the villages of Escolca and

Lotzorai (Sardinia, Italy). Journal of Herbs and Spices Medecinal Plants 2005; 11, 67-84.
13. Aboul-Enein AM, Abu El-Ela F, Shalaby EA, El-Shemy HA. Traditional medicinal plants research in Egypt: Studies of antioxidant and anticancer activities. Journal of Medicinal Plants Research 2012; 6 (5): 689-703.
14. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H Phytochemical screening and Extraction: A Review Internationale pharmaceuticasciencia 2011; 1 (1): 98-106.
15. Tuzlaci, E. Revision of the genus Asphodeline (Liliaceae)- a new infrageneric classification. Candollea 1987; 42: 559–576.
16. Marino A, Zengin G, Nostro A, Ginestra G, Dugo P, Cacciola F, Miceli N, Maria F, Angela F, Giuseppe B, Abdurrahman A. Antimicrobial activities, toxicity and phenolic composition of *Asphodeline anatolica* E. Tuzlaci leaf extracts from Turkey. Natural Product Research 2016; 4:1-4.
17. Zengin G. Aktumsek A, Guler GO, Cakmak YS, Girón-Calle J, Alaiz M, Vioque J. Nutritional quality of protein in the leaves of eleven *Asphodeline* species (Liliaceae) from Turkey. Food Chem. 2012;135:1360–1364.
18. Ali-Shtayeh MS, Yaghmour RM, Faidi YR, Salem K, Al-Nuri MA Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J of Ethnopharmacology 1998; 60:265–271.
19. Lazarova IE, Marinova G, Todorova-Nikolova I, Kostova A. Antioxidant properties of *Asphodeline lutea* of Bulgarian origin. Rivista Italiana Delle Sostanze Grasse 2009; 86(3):181-188.
20. Gulfraz M, Imran M, Khadam S, Ahmed D, Asad JM, Abassi KS, Irfan M, Mehmood S. A comparactive study of antimicrobial and antioxidant activities of garlic (Allium sativum L.) extract in various localities of Pakistan. African Journal of Plant Science 2014; 8(6) :298-306.
21. Celiktas OY, Kocabas EEH, Bedir E, Sukan FV, Ozek T and Baser KHC, 2007- Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chemistry*, 100: 553-559.
22. Datta P, Gulati N, Singla N, Vasdeva HR, Bala K, Chander J, Gupta V. Evaluation of various methods for the detection of meticillin-resistant *Staphylococcus aureus* strains and susceptibility patterns. Journal of Medical Microbiology 2011; 60, 1613–1616.
23. National Committee for Clinical Laboratory Standards (NCCLS). 2013- Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Pennsylvania: NCCLS*; p*. M100-S23.
24. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M, Khan AU, Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin Molecules 2009; 14, 586-597.
25. Perumal S, Pillai S, Cai LW, Mahmud R, Ramanathan S. Determination of minimum inhibitory concentration of *Euphorbia hirta* (L.) extracts by tetrazolium microplate assay. Journal of Natural Product 2012; 5:68–76.
26. El-Seedi HR.Antimicrobial arylcoumarins from *Asphodelus microcarpus*. Journal of Natural product 2007; 70: 118-120.
27. Ghoneim MM, Elokely KM,. El-Hela AA, Mohammad AI, Jacob M, Radwan MM, Doerksen RJ, Cutler SJ, Ross SA. Asphodosides A-E, anti-MRSA metabolites from *Asphodelus microcarpus*. Photochemistry 2014; 105: 79–84.
28. Nelson K, Lyles JT, Li T, Saitta A, Addie-noye E, Tyler P and Quave CL. Anti-acne activity of Italian medicinal plants used for skin infection. *Front. Pharmacol.*2016; 7:425.
29. Uysal A, Lazarova I, Zengin G, Gunes E, Aktumsek A, Gevrenova R. New Perspectives on *Asphodeline lutea* from Bulgaria and Turkey: Anti-mutagenic,Anti-microbial and Anti-methicillin Resistant *Staphylococcus aureus* (MRSA) Activity.Bri J of Pharma Res 2016; 10(3): 1-10.
30. Abuhamdah S, Abuhamdah R , Al-Olimat S, Chazot P. Phytochemical Investigations and Antibacterial Activity of Selected Medicinal Plants from Jordan. European Journal of Medicinal Plants 2013; 3(3): 394-404.
31. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol 1998; 60: 1-8.
32. Ncube NS, Afolayan AJ, Okoh, AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7 (12):1797-1806.
33. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Intern Pharma 2011; 1(1): 98-106.
34. Jaffery E, Brown A, Kurilich A, Keek A, Matusheski N, Klein B. Variation in content of bioactive components in broccoli. J Food Compos Anal. 2003; 16:323–30.
35. Rafat A, Philip K, Muniandy S. Antioxidant potential and content of phenoliccompounds in ethanolic extracts of selected parts of *Andrographis paniculata*. Journal of Medical plants research 2010; 4:197–202.
36. Cowan MM Plant products as antimicrobial agents. Clinical Microbiology Review 1999; 12: 564-582.
37. Gibbons S. Anti-staphylococcal plant natural products. Natural Product Reports; 2004 21: 263-277.
38. Todorova NG, Lazarova I, Mikhova B, Kostova I. Anthraquinone, naphthalene and naphthaquinone components of *Asphodeline lutea*. Chemistry of Natural Compounds 2010;46:322–323.
39. Weia Y, Qi L, Jia Y, Qiang F, Ling Z, Huiping S, Wang W Antibacterial mode of action of 1,8-dihydroxy-anthraquinone from *Porphyra haitanensis* against *Staphylococcus aureus* .Natural Product Research 2014; 8(1):1-4.