Effect of bioactive compounds of *Artemisia herba alba* on some bacteria isolated from El-Manzala water treatment plant

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Abstract: *Artemisia*, one of the most genera in the family Asteraceae and the largest genus in the tribe Anthemideae, comprises from 200 to more than 500 taxa at the specific or subspecific level. Many Artemisia species have a high economic value in several fields, as antimicrobial agents in pharmacy, food plants and as antihelminthic and antimalaria in medicine. Artemisia herba-alba was known for its therapeutic and medicinal properties, it was used in both traditional and modern medicine. Several papers have been published on the chemical composition of specimens of Artemisia herba-alba. The aim of this work is to throw light about significance of *Artemisia herba alba* extracts through GC-MS analysis and determining the major derivatives of *Artemisia herba alba* bioactive compounds.

[Haroun B.M., Elghamry A.A., Abdel-Shakour E.H., Elmorsy S.M. and Zahem Y.H. **Effect of bioactive compounds of *Artemisia herba alba* on some bacteria isolated from El-Manzala water treatment plant.** *Rep Opinion* 2014;6(2):59-64]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 8

Keywords: *Artemisia herba-alba*; Biological activities; Antimicrobial activity; Agar well diffusion method; GC-MS analysis

1. Introduction

The genus *Artemisia* related to family *Asteraceae*. It includes a variable number of species (from 200 to over 400) found throughout the northern half of the world (Barberá and Marco, 1990). *Artemisia herba-alba* Asso is a shrub growing wild in semi- arid or arid areas all around the Mediterranean basin. This plant is widespread from Southeastern Spain to Turkmenistan and Uzbekistan and in Near East and North Africa (Quezel and Santa, 1962; Nabli, 1989).

Artemisia herba-alba, known also as desert wormwood (known in Arabic as shih) Herbal tea from this species has been used as analgesic, antibacterial, antispasmodic, and hemostatic agents (Laid et al., 2008). During an ethnopharmacological survey carried out among the Bedouins of the Negev desert, it was found that *Artemisia herba-alba* relieved stomach disorders (Friedman et al., 1986). This plant is also suggested to be important as a fodder for sheep and for livestock in the plateau regions of Algeria where it grows abundantly (Fenardji et al., 1974; Benmansour and Bendiab, 1998). Ascaridae from hogs and ground worms were killed by the oil of the Libyan *A. herba-alba* in a short time (Callegari and Rossi, 1939; Callegari and Rossi, 1940).

In the present study, the antimicrobial potency of chloroform, methanol extracts of *Artemisia herba-alba* were investigated by agar well diffusion method. 25 bacterial strains isolated from El-Manzala water treatment plant were used as test cultures. The antibacterial activity was determined by agar well diffusion method. The preliminary phytochemical screening was carried out to identify the derivatives in the extracts by using GC-MS technique.

The aim of this work: isolation of bacteria from El-Manzala water tretment plant, Purification and Identification of the isolated bacteria, Sensitivity testing for the isolated bacteria using *Artemisia herba alba* extract discs.

2. Materials and Methods

The plant extracts were prepared using the solvents water, methanol and chloroform. 10g of *Artemisia herba-alba* were taken and homogenized with 100ml of the respective solvents. The crude preparation was left overnight in the shaker at room temperature and then centrifuged at 4000rpm for 20mins. The supernatant containing the plant extract was then transferred to a pre-weighed beaker and the extract was concentrated by evaporating the solvent at 60°C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide, to obtain a final concentration of 20mg / 5µl.Finally plants extracts were tested on bacterial isolates using Paper disc method.

2.2. Water samples

Samples of raw water before entering El-Manzala water treatment plant from different distances, as well as from treated water sample emerging from the treatment plant were collected in a clean, sterile glass bottles. The bottle is filled with raw water sample by placing it under water surface and closed immediately as soon as filled with adequate water sample. Then transferred to laboratory preserved in ice box as soon as possible.

2.3. Bacterial isolation, purification and identification

It was performed by inoculating 1.0 ml of primary effluent of each water samples and streaking on the Nutrient, MacConkey and Blood agar medium. Bacterial isolates were purified using streak method on nutrient, MacConkey and Blood agar medium. The purification procedure of the bacterial isolates under investigation was carried out by the agar streak plate method. Colonies of different morphological forms were picked up and re streaked on the surface agar of plates containing the same isolation medium. After incubation for 24 hours, separate colonies of distinct shape and color were picked up and re streaked separately several consecutive times onto the surface of agar plates containing the isolation media to assure purity (Ugwa1 et al., 2013). Purity was checked up microscopically and morphologically using Gram stain. Pure isolates only were sub cultured on slants of the isolation medium and kept for further investigation. The purified colonies were prepared to be used for a complete biochemical identification using a VITEK 2 Compact system for bacterial identification.

2.4. Preparation and extraction of plant material

The plant extract (Leaf and Stem) was prepared using the solvents water, methanol and chloroform. 10g of the samples were taken and homogenized with 100ml of the respective solvents. The crude preparation was left overnight in the shaker at room temperature and then centrifuged at 4000 rpm for 20 mins. The supernatant containing the plant extract was then transferred to a pre weighed beaker and the extract was concentrated by evaporating the solvent at 60°C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide, finally plants extract was tested on bacterial isolates using agar well diffusion method.

2.5. Antimicrobial paper disk method

Analytical paper discs (6.0 mm in diameter) were saturated with *Artemisia herba-alba* extract and aseptically placed on the surface of the inoculated plates seeded with different bacterial isolate separately (Steers et al., 1959; Iakushkina et al., 1988) .

2.6. GC/MS analysis

The qualitative and quantitative composition of the plant extract were studied by Gas Chromatography and Mass Spectroscopy (GC-MS) analysis. A Perkin-Elmer gas chromatograph (model 8700), equipped with a flame ionization detector (FID) and HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm) was used for the chemical analysis of the essential oils. Injector and detector temperatures were set at 220 and 290^{o}C, respectively. The column oven temperature was programmed from 80^{o}C to 220^{o}C at the rate of 4^{o}C/ min; initial and final temperatures were held for 3 and 10 minutes, respectively. Helium was used as a carrier gas with a flow of 1.5 mL/ min. A sample of 1.0 µL was injected, using split mode (split ratio, 1:100). A built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin-Elmer, Norwalk, CT, USA) was used for quantification purposes.

2.6.1. Identification of compounds

Identification of compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library data GC-MS system (wiley 229). The relative percentage of each compound obtained according to it's under peak area in GC chromatogram, without the use of correction factors.

3. Results and Discussion

Isolation of bacterial isolates was performed by inoculation of water samples on nutrient agar, MacCkonkey's and blood agar plates respectively. Results indicates that a total of 63 bacterial isolates could be isolated from 4 different climatic conditions at four consecutive times (Spring, Summer, Autumn and Winter seasons).

All bacterial isolates obtained during spring, summer, autumn and winter seasons were identified on a VITEK® 2 compact system for bacterial identification. The VITEK® 2 compact system is a fully automated system that performs bacterial identification by biochemical analysis using colorimetry. The VITEK® 2 compact system for bacterial identification is highly automated that allows for the rapid and accurate identification of some bacterial strains. In total, the system's database is capable of identifying a variety of microorganisms.

All the total identified bacterial isolates 63 isolates belong to only 25 water bacterial species as follow: *Bacillus cereus*; *B. brevis*; *B. coagulans*; *B. firmus*; *B. megaterium*; *B. mycoides*; *B. pumilus*; *Staphylococcus gallinarum*; *Staphylococcus haemolyticus*; *Staphylococcus capitis; Staphylococcus vitulins; Staphylococcus sciuri*; *Enterobacter agglomerans*; *Enterobacter aewrgenes*; *Klebsiella oxytoca*; *Edwardsiella ictalluri*; *Citerobacter freundii*; *Salmonella pullorum*; *Shigella dysenteriae*; *Shigella flexneri*; *Providencia rettgeri*; *Proteus rettgeri*; *Micrococcus lutes; Sphingomonas paucimobilis*; and *Escherichia coli*, as shown in table (1).

Table (2) showed that 21 bacterial isolates including *Bacillus cereus*; *Bacillus brevis*; *Bacillus coagulans*; *Bacillus megaterium*; *Bacillus mycoides*; *Bacillus pumilus*; *Staphylococcus gallinarum*; *Staphylococcus haemolyticus*; *Staphylococcus capitis; Staphylococcus vitulins; Staphylococcus sciuri*; *Enterobacter agglomerans*; *Klebsiella oxytoca*; *Citerobacter freundii*; *Salmonella pullorum*; *Shigella dysenteriae*; *Shigella flexneri*; *Providencia rettgeri*; *Proteus rettgeri*; *Sphingomonas paucimobilis*; and

Escherichia coli are sensitive to *Artimizia* extract when extracted with Methanol:H_2O except *Edwardsiella ictalluri*; *Enterobacter aewrgenes*; *Micrococcus lutes* and *Bacillus firmus* which are resistant.

Table 1. Species isolated and their code number.

No.	Suggested name	Organism code no. and repeatability
1	*Micrococcus lutes*	33-53-55
2	*Edwardsiella ictalluri*	1-31-43
3	*Staphylococcus haemolyticus*	2-6-8-57
4	*Shigella dysentreriae*	3-4-62
5	*Klebsiella oxytoca*	5
6	*Bacillus megaterium*	7-9-18-23
7	*Bacillus firmus*	58
8	*Staphylococcus sciuri*	42-47-50
9	*Staphylococcus gallinarum*	20-26-29
10	*Staphylococcus capitis*	49-52-61
11	*Bacillus pumilus*	15-35-54
12	*Bacillus coagulanse*	19-22-25
13	*Staphylococcus vitulens*	59
14	*Salmonella pullorum*	36
15	*Citerobacter freundii*	46-51-56
16	*Shigella flexneri*	48-60
17	*Bacillus brevis*	32-34
18	*Bacillus mycoides*	11-12-16-63
19	*Enterobacter agglomerans*	28-39-44
20	*Proteus rettgeri*	21-38-40
21	*Providencia rettgeri*	27-30
22	*Enterobacter aewrogenes*	10-13
23	*Escherichia coli*	41-45
24	*Sphingomonas paucimobilis*	14-17
25	*Bacillus cereus*	24-37

Twenty bacterial isolates including *Bacillus cereus*; *B. brevis*; *B. coagulans*; *B. firmus*; *B. megaterium*; *B. mycoides*; *Staphylococcus gallinarum*; *Staphylococcus haemolyticus*; *Staphylococcus capitis; Staphylococcus vitulins; Enterobacter agglomerans*; *Enterobacter aewrgenes*; *Klebsiella oxytoca*; *Salmonella pullorum*; *Shigella flexneri*; *Providencia rettgeri*; *Proteus rettgeri*; *Sphingomonas paucimobilis*; and *Escherichia coli species* are sensitive to *Artimizia* extract when extracted with Methanol: Chloroform except *Edwardsiella ictalluri*; *Citerobacter freundii*; *Staphylococcus sciuri*; *B. pumilus* and *Micrococcus lutes* which are resistant.

The above explains that the methanolic water extract was better than the methanolic chloroform extract in affecting large number of organisms as observed from the effect and difference in diameter of clear zone between the two extracts. Similar results were obtained by Benli et al. (2007), their results indicated that the methanol extract of *Artimizia dracunculus* was more effective against tested microorganisms than chloroform or acetone extracts. The chloroform and acetone extracts were inhibitory only towards *Pseudomonas aeruginosa* (ATCC 27853). While the methanol extract that was diluted with 10 ml distilled water showed inhibition zones against *Shigella* (RSHI), *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* (ATCC 27853), the methanol extract that was diluted with 5 ml distilled water showed inhibition zones against two different strains of *Escherichia coli* (RSHI, ATCC 25922), *Shigella* (RSHI), *Listeria monocytogenes* (ATCC 7644), and *Pseudomonas aeruginosa* ATCC 27853.

Our results were better than that reported by Anjali et al. (2009) in that both methanolic and chloroform extracts have antimicrobial activity against a wide range of bacteria. Anjali et al. (2009) reported that methanolic and chloroform extracts were analyzed for their antibacterial capacity against six bacterial strains and an yeast strain. The antibacterial activity was determined by using disc diffusion method. *Bacillus cereus* was found to be more susceptible strain. Only methanol extract of *Artemisia herba alba pallens* showed the activity. Therefore, this was selected in their study for further investigation to determine its therapeutic potential.

On analysis by GC-MS, several compounds were identified from *Artimizia* extract and tabulated (Table 3). The chromatogram is shown in figure (1). In this study, GC-MS analysis revealed the presence of 20 compounds Cyclotetrasiloxane, octamethyl-, Benzaldehyde, 3-methoxy-4-[(trimethylsilyl)oxy]-, O-methyloxime, Pyridine, 3-(1a,2,7,7a-tetrahydro-2-methoxy-1-phenyl-1,2,7-metheno-1H-cyclopropa[b]naphthalen-8-yl)-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- , 2-Aziridinone, 1-tert-butyl-3-(1-methylcyclohexyl)- , Naphthalene, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 2-Methoxy-4-vinylphenol, 1-Octyn-3-ol, 4-ethyl-,Ethanone, 1-(2-hydroxyphenyl)-, 1-Bromo-3-(2-bromoethyl)heptane, Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester, Octane, 1,1'-oxybis-,Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-, 1-Hexadecanol, n-Hexadecanoic acid, Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-, à-Santonin, Limonene epoxide and Stearic acid. 1-Bromo-3-(2-bromoethyl) heptane (15%), Octane, 1,1'-oxybis- (11%), Decane, 5,6-bis (2,2-dimethylpropylidene)-, (E,Z)- (10%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (8.3%) and à-Santonin (7.5%) were the compounds showing higher area%. In our study 1-Bromo-3-(2-bromoethyl)heptane (15%) was the highest abundant compound as shown from both peak area in table (3) and hight of peak in figure (1), followed by Octane, 1,1'-oxybis- (11%).

Table 2. Antimicrobial activity of tested *Artemisia herba alba* extract against bacteria.

	Plant extract / Organisms	Methanol:H_2O extract (10:90 %)	Methanol:Chloroform extract (50:50 %)
		Inhibition zone (mm)	
1	*Staph. haemolyticus*	10	10
2	*Edwardsiella ictalluri*	R	R
3	*Bacillus mycoides*	20	12
4	*Klebsiella oxytoca*	14	18
5	*Citrobacter fruendii*	19	R
6	*Bacillus brevis*	25	13
7	*Bacillus coagulanse*	19	20
8	*Protus rettgerri*	20	20
9	*Staphylococcus sciuri*	17	R
10	*Shigella flexneri*	19	15
11	*Enterobacter aewrogene*	R	10
12	*Shigella dysintrie*	17	13
13	*Staph. gallinarum*	16	15
14	*Bacillus cerus*	19	15
15	*Micrococcus lutes*	R	R
16	*Bacillus megterium*	19	15
17	*Enterobacter agglomeranse*	20	13
18	*Salmonella pullorum*	18	13
19	*Providinica rettegri*	16	13
20	*Bacillus pumiles.*	18	R
21	*Staph. capitis*	20	22
22	*Sphingomonas paucimobilis*	19	13
23	*Staph. vitulinus*	17	10
24	*Bacillus firmus*	R	13
25	*E.coli*	22	17

Table 3. The separated bioactive compounds from *Artimizia herba alba* extract using GC-MS analysis.

Peak number	Retention Time RT (min)	Area %	Chemical Name	Molecular weight (Mwt)
1	6.0	2.32	Cyclotetrasiloxane, octamethyl-	296
2	8.0	1.51	Benzaldehyde, 3-methoxy-4-[(trimethylsilyl)oxy]-, O-methyloxime	151.1
3	16.0	2.36	Pyridine, 3-(1a,2,7,7a-tetrahydro-2-methoxy-1-phenyl-1,2,7-metheno-1 H-cyclopropa[b]naphthalen-8-yl)-	260
4	18.0	1.33	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.1
5	19	6.50	2-Aziridinone, 1-tert-butyl-3-(1-methylcyclohexyl)-	209
6	22	6.5	Naphthalene	128.1
7	26	8.3	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	126
8	27	2.16	2-Methoxy-4-vinylphenol	150.1
9	28	2.15	1-Octyn-3-ol, 4-ethyl-	145
10	32	4.2	Ethanone, 1-(2-hydroxyphenyl)-	284
11	**33**	**15**	**1-Bromo-3-(2-bromoethyl)heptane**	**193**
12	36.3	2.5	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	136
13	**38**	**11**	**Octane, 1,1'-oxybis-**	**242**
14	39	2.3	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-	348
15	39.5	5.1	1-Hexadecanol	242
16	40	5.0	n-Hexadecanoic acid	256
17	**41**	**10**	**Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-**	**333**
18	42	7.5	à-Santonin	246
19	43.5	1.4	Limonene epoxide	152
20	48	2.33	Stearic acid	284

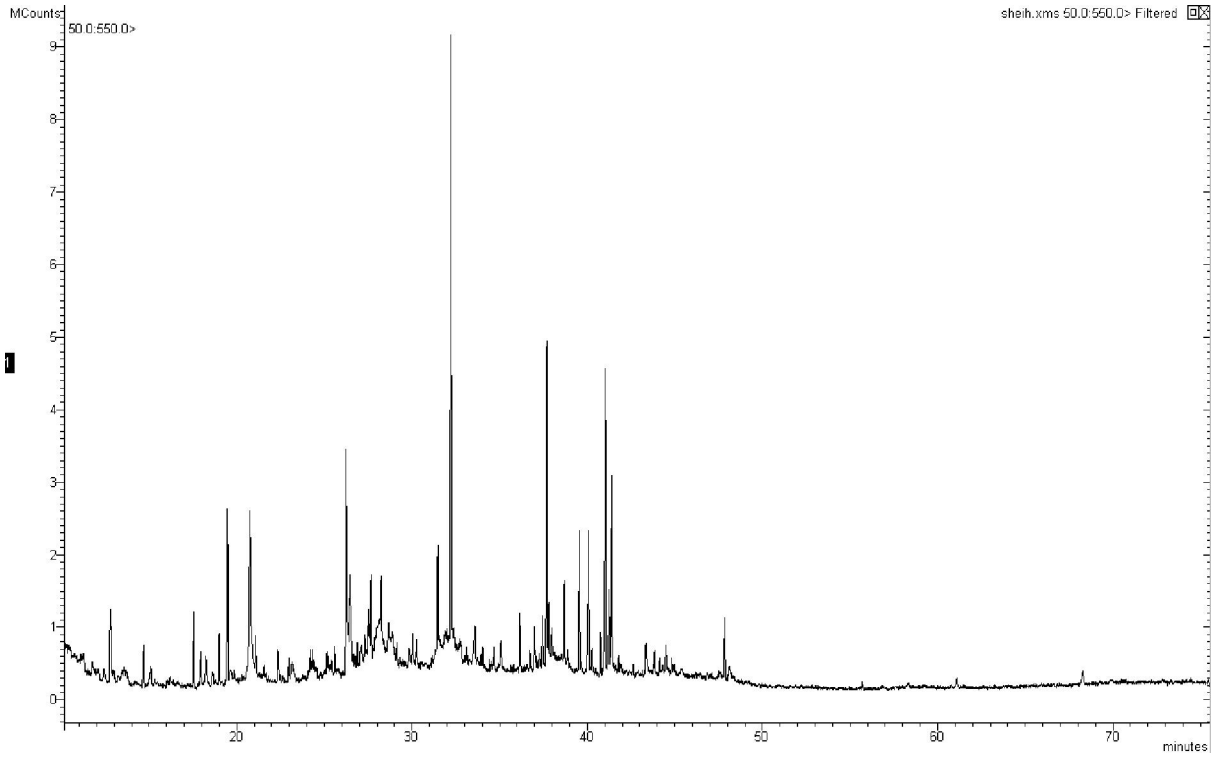

Figure 2. The chromatogram of all compounds produced from GCMS analysis of *Artimizia herba alba* extract.

4. Conclusion

This paper studied antimicrobial activities of the bioactive compounds of *Artimizia herba alba* extract against bacteria isolated from El-Manzala water treatment plant, *Artimizia herba alba* extract is a good source of antibacterial compound, different extraction methods and different solvents will elute different bioactive and this is agood idea to apply natural products of some desert plants as antimicrobial agents in the future at large scale.

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References

1. Anjali DR, Gayatri SK, Vedavati GP and Nirmala RD (2009). Antimicrobial Screening of Medicinal Plant –Artemisia pallens. International Journal of Pharm. Tech Research 1(4): 1164-1166.
2. Barberá O and Marco JA (1990). Natural products from the genus Artemisia L. In: Atta-ur- Rahman (Ed.). Studies in Natural Products Chemistry, vol. 7. Elsevier Science Publishers BV, Amsterdam pp. 201-264.
3. Benli M, Kaya I and Yigit N (2007). Screening antimicrobial activity of various extracts of *Artemisia dracunculus L.* Cell Biochem. Funct., 25(6):681-686.
4. Benmansour A. and taleb Bendiab S. A. (1998). Comparative investigation of proteins and amino acids in *Artemisia herba-alba* residues and Algerian date stones. Proposal to use them as additional feed for livestock. *J. de la Societe Algerienne de Chimie*, 8(1): 67-71.
5. Callegari L and Rossi A (1939). The active principles of Lybian *Artemisia herba-alba* and their pharmacological action. *Archivio Italiano di Scienze Farmacologiche*, 8: 145-160.
6. Callegari L and Rossi A (1940). The active principles of Lybian *Artemisia herba-alba* and their pharmacological action. *Chimie et Industrie (Paris)*, 44, 321.
7. Fenardji F, Klur M, Fourlon C and Ferrando R (1974). White *artemisia*, (*Artemisia herba-alba*), *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 27(2):203-206.
8. Friedman J, Yaniv Z, Dafni A and Palewitch D (1986). A preliminary classification of the healing potential of medicinal plants, based on a rational analysis of an ethnopharmacological field survey among Bedouins in the Negev desert, Israel. *J. of Ethnopharmacol.*, 16(2-3): 275-287.
9. Iaksuhkina IV, Malkova IV and Prozorova IN (1988). Study of the activity of a new

glycopeptide antibiotic eremomycin combined with to bramyrin against *Staphylococciinvitro*. Antibiot. Khimioter. 33 (5): 342-345.
10. Laid M, Hegazy M. F , Ahmed A. A, Ali K, Belkacemi D and Ohta S (2008). Sesquiterpene lactones from Algerian *Artemisia herbaalba*. *Phytochemistry lett.*, 1: 85-88.
11. Nabli A. (1989). Essai de synthèse sur la végétation et la phyto-écologie tunisienne. *Programme flore et végétations tunisiennes*, volume 4 A 6. p 193.
12. Quezel P and Santa S (1962) . *Nouvelle flore de l'Algérie et des régions désertiques méridionales*. Tome I. Édit. CNRS, Paris, p 565.
13. Steers E, Foltz EL and Graves BS (1959). An inoculate replication apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. 9: 307 - 311.
14. Ugwa1 IK, Ukponu AF, Omorusi1 VI and Igeleke CL (2013). Biodegradation of Gamalin-20 by Micrococcus sp (Strain 189) in the Coastal Soils of Southeastern Nigeria. Nature and Science, 11(5).

2/13/2014