Original Article

Photochemical Composition and Antimicrobial of Essential Oils from Two Artemisia Species for their Application in Drinking-Water

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ABSTRACT

Background: This research intends to analyze the essential oil composition and antimicrobial activity of the two *Artemisia* species from the Sistan and Baluchestan region of Iran. The aerial parts of *A. santolina* Schrenk and *A. sieberi* Boiss were collected during the flowering stage.
Methods: The essential oils were isolated by the hydro distillation method. The (w/w) oils yield were 0.84 % and 1.29 % for *A. santolina* Schrenk and *A. sieberi* respectively. The oils constituents were analyzed by the GC-MS technique.
Results: In the oil of *A. santolina*, 67 constituents embracing 95.16 % of total essential oil were recognized. The main constituents were 1, 8-cineole (21.07 %), camphor (13.13 %) and unknown constituents (4.84 %). In the oil of *A. sieberi* 72 constituents were recognized consisting of 96.49 % of its total essential oil. The main constituents were 1, 8-cineole (45.88%), camphor (3.40%) and unknown constituents (3.51%).
Conclusion: The findings reveal that the *A. sieberi and A. santolina* essential oils have a considerable percentage of oxygenated terpenoids with the anti-microbial property. The antimicrobial effects of essential oil have been demonstrated in drinking-water using the heterotrophic plat technique.

Keywords: *Artemisia santolina* Schrenk, *Artemisia sieberi* Boiss, Hydrodistillation method, GC-MS technique, Heterotrophic plat count method.

INTRODUCTION

The genus "*Artemisia"* (Composite), with the common Persian name of "Dermaneh", includes 34 species that are found wild all over Iran[1]. *A. sieberi* is a shrubby, perennial land rough plant, with the height of 30-50cm spider web villies, and stems full of flowers straight rising from base. Leaves are oval and petiolate. Having petioles and full of rectangular banicule flowers, narrow and nearly hyacinth from.

The flowers are apart from each other with hyacinth shape Perennial land rough plant, with the height of 30-50cm spider web villies, and stems full of flowers straight rising from base. Leaves are oval and petiolate. Having petioles and full of rectangular banicule flowers, narrow and nearly hyacinth from. The flowers are apart from each other with hyacinth shape[2,3]. This plant grows in northern part of the Taftan mountain, the valley whit a height of 2100m, and the valley of Tamondan which is a of stony place, full of steeps and heights, on average more than 40 % slope. It has dry and cold climates with average annual rain fall rate of 300-400 mm, the precipitations usually occur in the form of snow fall. This plant grows in soils with light texture, low amount lime, without saltiness, and with a small rate of alkali, pH 7.4-7.7, which is considered relatively poor, regarding organic materials[4,5]. Chemical analyze of the soil of growing place of two species are Carbon rate of 0.54 % for *A.santolina* and 0.27 % for A. *sieberi*. The result shows that soil slope, regarding amount of Jomos is poor. Amount of metal ions Mn, Zn, Fe is less than those of forest soil but Ca, Mg, Si are more than those of forest soil[6].

A. santolina is a rough bush with a 35-45 cm height and its wreath of flowers has a diameter of 20-40 cm. It has various stems with white felt villies, its leaves are relatively thick with grey villies and flowers are mixed binnacle. It is wide-spread in heights of 1500m. It grows in light-textured soil, without saltiness and with low alkali environment (pH 7.4-8.8) which is poor regarding organic materials. Average annual rain fall needed for the plant is 150-250 mm[7]. In this research, we intend to identify the constituents of essential oil associated with these two- very important species, *A. sieberi* and *A. santolina* and examine the antimicrobial property.

Farzaneh *et al*[8] were studied *A. sieberi* is widely distributed in desert area of Iran. The aerial parts of *A. sieberi* were collected from Bajestan (Khorasan province) at flowering stage. The essential oil was obtained by hydro-distillation of air-dried samples and the chemical composition identified by GC-MS. Oxygenated mono-terpens were the major components of the oil: β-thujone (19.8%), α-thujone (10.5%), comphor (19.5%), verbenol (9.7%), p-mentha-1, 5-dien-8-ol (6.4) and davanone (5.8%).

Negahban *et al*[9] investigated *Artemisia sieberi* is a widely distributed plant in Iran. Because some species of *Artemisia* are insecticidal, experiments were conducted to investigate fumigant toxicity of the essential oil. Dry ground leaves were subjected to hydro distillation using a modified Clevenger-type apparatus and the resulting oil contained camphor (54.7%), camphene (11.7%), 1, 8-cineol (9.9%), *β*-thujone (5.6%) and *α*- pinene (2.5%).

Weyerstahl *et al*[10] the essential oil of the aerial parts of *Artemisia sieberi* Bess has been investigated by a combination of chromatographic and spectroscopic methods. This oil possesses a fresh herbaceous, camphoraceous, earthy odour with a fruity and dried plum-like background. The main constituents of the essential oil are comphor (44%), 1, 8-cineole (19%), camphene (5%), terpinen-4-ol (2.5%), α-terpineol (2%). The major

component of the sesquiterpenes (altogether only 4.5% of the oil) is dehydro-1, 8-sesquicineole.

Sefidkon *et al*., (2002) worked on *A. santolina* in central Iran and showed the main components of the *A. santolina* oil as 1, 8-cineole (6.50%), trans-verbenol (9.90%), neryl acetate (13.40%), bornyl acetate (10.90%), lavendulol (8.80%) and linalool (6.9%)[11].

Demirci *et al*[12] studied *A. santolina* collected from the Antalia area of Turkey. Essential oil of this plant with a yield of 1.60% w/w was extracted, and 71 constituents were finally recognized forming 91.16 % of total oil. The most important constituents included ketone (38.10%), comphor (11.70%), β-phellandrene (9.2%), β-pinene (2.10%), terpinolene (1.60%), yomogi alcohol (1.50%) and Artemisia alcohol (1.50 %).

The study of *A. santolina* from the Corse region of France showed that its mian oil constituents included 1, 8-cineole (2.00%), terpinolene (1.00%), terpinen-4-ol (1.40%), myrcene (34.60%), β-phellandrene (11.70%)[13]. The present study deals with the identification of the constituents of essential oils of *A. sieberi* and *A. santolina* and its antimicrobial property.

MATERIALS AND METHODS

Plant materials

The aerial parts *A. santolina* Schrenk and *A. sieberi* .Boiss were collected during the flowering stage from the heights of the Taftan Mountain and Tamondan valley in May, 2008. The collected plants were dried and ground into powder and stored until use.

Optimizing the conditions for extraction of essential

It is necessary to determine the proper time and quantity of plant samples, as well as distillation-flask volume and water amount in two time-distillations for providing needed vapor intensity to carry out the stream distillation. 50 g of the ground plant sample (*A. sieberi*) was mixed with different volume of water for two time-distillations in a distillation-flask (one liter capacity). The extraction process was done for a period of 3.5 h and was repeated in distillation-flask of two capacities for optimization of good yield (Figure.1).

Isolation of essential oil

Fifty g sample of the prepared powder was put in a two-liter distillation-flask with 300 ml water and was extracted in Clevenger's apparatus twice for a period of 3.5 h. Obtained essential oil was collected in n-hexane solvent and water. The purified essential oil was stored in a sample container at 4^0C until the analysis of oil constituents using GC-MS.

GC-MS analysis

The analysis of essential oils was performed using a GC System, equipped with a HP-5Ms capillary column, (0.25mm id, 0.25µm) and a HP 5973 mass selective detector in the electron impact mode. Helium was the carrier gas at 1 ml/min .Injector and MS transfer line temperature was at 250^0C and 260^0C respectively. Column temperature was set at 40^0C for 1 min, then programmed from 40^0C to 250 ^{0}C at a rate of 3^0C/min and finally held isothermally for 20 min. For GC-MS detection an electron ionization system was used with ionization energy of 70 eV.

Retention indices were calculated by using retention times of C_8-C_{26} that were injected after the oil at the same chromatographic conditions[14]. The linear retention indices for all the compounds were determined by injection of the sample with a solution containing a homologous series of C_8-C_{26} n-alkanes. The individual constituents were identified by their identical retention indices, referring to

known compounds from the literature[15] and also by comparing their mass spectra with either the known compounds or with the Wiley (7th edition).library mass spectral database.

Heterotrophic plat count method

The Heterotrophic plat Count (H.P.C), represents an indication of aerobic and anaerobic bacteria that derive their energy and carbon from organic compounds. The number of these bacteria depends on the composite of culture environment, ancubation period (1-7 days) and temperature of incubation (20-35^{0}C). This group consists of hot-negative bacteria belonging to the following species: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Aspergillus niger, Alkali genes, Klebsiella* and intro *bacteria-moraxela.* H.P.C bacteria in drinking water is variable and mostly Under the influence of temperature, remained chlorine existence and concentration of absorbable organic materials. The amount of H.P.C should not exceed 500 organisms/ml. The H.P.C is a useful parameter to evaluate water quality in distribution systems and water filtering house[16].

RESULTS

Essential oil of two species of *Artemisia* (*A. santolina* and *A. sieberi*) was extracted using the hydro distillation method. The ratio of extract yield (w/w) has been 0.84% for *A. santolina* and 1.29% for *A. sieberi* using GC-MS technique. 67 constituents amounting 95.16 % of the total essential oil was identified in *A. santolina* (Table 1). The most important constituents of this specie included 1, 8-cineole (21.07%), comphor (13.13%), chrysanthenone (6.98 %), trans-methyl cinnamate (5.56%), lyratyl acetate (5.20%), 4-terpineol (4.39%), and borneol (3.75%). 69.73% of these components consisted of oxygenated terpenoid and unknown compounds were only 4.84 %.

Essential oil of *A. sieberi*, 72 components was recognized comprising 96.49 % of total essential oil. The main constituent (Table 2) included 1, 8 cineole (45.88%), 4-terpineol (3.89%), camphor (3.40%), chrysanthenone (3.00%), α-terpineol (2.97%), methyleugenol (6.44%) and eugenol (2.75%). 72.04 % of these constituents consisted of oxygenated terpenoid and 3.51% were unknown constituents (Table 3). The effective essential oil concentration for its anti-microbial property has been tested in the H.P.C test with 0.1, 0.3, 0.5, 0.7, 1.1(ml) and 1.5 ml concentration for *A. sieberi* oil (Figure 2) and *A. santolina* oil (Figure 3). An antimicrobial test was performed using the H.P.C method which ascertained the antimicrobial property (dominance of oxygenated components) in essential oils extracted from these plants.

DISCUSSION

In essential oils of *A. santolina* and *A. sieberi*, 29 similar compounds with a high percentage were recognized. In the essential oil of *A. santolina* was more than 76 % terpenoids' increase resulted in better quality of the essential oil. Oxygenated terpenoids' percentage in essential oil of *A. sieberi* is considerably more than essential oil of *A. santolina.*

Anti microbial test was performed by method of Heterotrophic plate count (H.P.C) regarding *A. sieberi*, and *A. santolina*. The result showed completely the property of antimicrobial that confirms dominance of oxygen-having components in essential oil of above plant. According to Figures 2 and 3, this essential oils can be used in microbiologic filtration of drinking water Microorganisms in the lowest volume (1.1 ml) were bound by essential oil of *A. sieberi.*

Microorganisms in the highest volume (1.5 ml) were bound by essential oil *A.*

Santolina. In addition, in our research work; plenty of various species of *Artemisia* genus in this Region have plentiful essential oil such as cineole and camphor which can be utilized in pharmacy industry.

CONCLUSION

Since these two plants were collected from two different places with short distance in the area of Taftan, Baluchestan Iran, and the influence of different climate and soil features was easily recognizable on their growth and evolution, as well as on the quantity of effective substances of the plants. Both the plants were extracted and resultant essential oils showed an acceptable level of quality. Essential oil of *A. santolina* had higher quality and percentage of the terpenoid constituent than that of *A. sieberi.* The constituent of 1,8 cineole that is the most common component in essential oils after camphor was found in good amount in both the plants (of higher quality) and in widely used in the production of antiseptic and anesthetic medicines[17].

Camphor constituent which is a antiseptic and is applied in the chemistry of rubber and paper as well as in hygienic and cosmetic materials, was also found in both the plants in a considerably good amount. In essential oils from both the plants, there were oxygen-containing terpenoid constituents. Anti-microbial experiments suggested that essential oils from these two plants could be used in the filtration of water.

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Table 1. Chemical composition of essential oil from *A. santolina*

S. No.	Compound	Retention indices	Percentage
1	α-pinene	904	0.05
2	Camphene	920	0.25
3	Verbenene	925	0.04
4	Benzaldehyde	931	0.11
5	3-methyl-2-β -butenalbbut	933	0.04
6	Sabinene	944	0.07
7	Hexanal	947	0.05
8	β-pinene	949	0.09
9	2, 3-dehydro-1, 8-cineole	962	0.81
10	1, 2, 3- triethylbenzene trimethylB benzene	965	0.09
11	Yomogi alcohol	969	0.24
12	Phellandrene	975	0.08
13	Amyl isobutyrate	980	0.21
14	α-terpinene	988	1.08
15	o-cymene	998	0.76
16	2-hexenal	1001	0.12
17	1, 8-cineole	1008	21.07
18	γ-terpinene	1030	1.47
19	Trans-sabinene hydrate	1040	0.89
20	Artemisia alcohol	1054	0.78
21	α-terpinolene	1060	0.45
22	Linalool	1072	0.43
23	cis-sabinene hydrate	1073	0.53
24	Filifolone	1077	1.34
25	Chrysanthenone	1101	6.98
26	1-terpineol	1118	0.98

27	Camphor	1126	13.13
28	Unknown	1133	2.44
29	Unknown	1139	0.95
30	Borneol	1145	3.75
31	4-terpineol	1155	4.39
32	p-cymen-8-ol	1160	0.31
33	β-fenchyl alcohol	1167	1.94
34	Unknown	1171	0.42
35	Myrtenol	1173	0.55
36	α-terpinene	1213	0.39
37	Trans-piperitol	1182	0.79
38	Carvone	1217	0.22
39	Geranial	1221	0.33
40	Pipertone	1228	0.60
41	Chrysanthenyl acetate	1230	0.22
42	Lyratyl acetate	1239	5.20
43	Triethylbenzene	1248	0.31
44	Bornyl acetate	1256	0.92
45	Thymol	1260	0.26
46	Carvacrol	1270	0.20
47	Cis-methyl cinnamate	1274	1.48
48	5, 5-dimethyl-1-ethyl-1, 3-cyclopentadiene	1291	0.48
49	Unknown	1322	1.03
50	Eugenol	1325	0.80
51	1-nonene	1335	0.29
52	Neryl acetone	1345	0.71
53	Trans-methyl-cinnamate	1357	5.56
54	2-ethylidiene-6 methyl-3, 5-heptadienal	1360	0.30
55	Methyleugenol	1369	1.45
56	Cis-jasmone	1371	1.22
57	5-ethyl m-xylene	1408	0.73
58	Homoadamantane	1437	0.62
59	E-ocimenone	1448	0.55
60	3-methoxy-2, 4, 6-trimethylphenol	1485	0.9 2
61	3-methoxy-2, 4, 6-trimethylphenol	1494	0.29
62	Spathulenol	1556	1.11
63	Caryophyllene oxide	1562	0.39
64	Isocitronellol	1580	0.33
65	1, 6, 9-tetradecatriene	1590	0.60
66	Trans-α- bergamotene	1598	0.34
67	α-pinene epoxide	1601	0.28
68	Methyl jasmonate	1613	0.21
69	1-(2, 3-dihydroxy-4-methoxy-6-methylphenyl) ethanone	1647	4.05
70	2, 6-dimethyl-2, 6-octadine-1, 8-diol	1680	0.50

	Diacetate		
71	Hexahydrofamesyl acetone	1797	0.05

Table 2. Chemical composition of essential oil from *A. sieberi*

S. No.	Compound	Retention indices	Percentage
1	3-methyl-3-buten-2-one	718	0.05
2	2-ethyl furan	742	0.01
3	3-methyl-1-butanol	767	0.02
4	Tricycle	903	0.02
5	α-pinene	908	0.09
6	Ethyl isobutyrate	917	0.02
7	Camphene	926	0.02
8	3-methyl-2-buten-1-ol	931	0.02
9	3-methyl-2-butenal	939	0.08
10	Sabinene	946	0.06
11	β-pinene	950	0.06
12	Hexanal	952	0.05
13	2, 3-dehydro-1, 8-cineole	963	0.49
14	1, 2, 3-trimethylbenzen	966	0.12
15	Heptanoic acid	973	0.06
16	Phellandrene	977	0.09
17	2-buten-1-ol acetate	978	0.01
18	Amyl isobutyrate	983	1.12
19	5-[1, 1-dimethylethyl]-1, 3-cyclopentadiene	987	0.02
20	α-terpinene	989	1.08
21	p-cymene	998	0.43
22	2-hexenal	1005	0.11
23	1, 8-cineole	1013	45.88
24	3-mthyl-2-cyclopentanone	1015	0.02
25	4-methyl-4-vinylbutyrate	1016	0.04
26	Benzene acetaldehyde	1018	0.02
27	2, 2, 9-trimethyl-trans-1, 6-dioxaspiro [4, 4] nonane	1028	0.54
28	Isoamyl acetate	1029	0.11
29	γ-terpinene	1032	1.34
30	Trans-sabinene hydrate	1041	0.82
31	Cis-linalool oxide	1045	0.16
32	α-terpinolene	1061	0.47
33	4, 7, 7- trimethylbicyclo [3.3.0] octan-2-one	1065	0.62
34	Isoamyl-2-methyl butyrate	1068	0.13
35	Linalool	1071	0.41
36	Cis-sabinene hydrate	1073	0.72
37	Filifolne	1076	0.58

38	N-hexyl isobutyrate	1079	0.09
39	4-methyl-2-pentenoic acid methyl ester	1086	0.07
40	1, 6-dimethyl hepta-1, 3, 5-triene	1098	044
41	Chrysanthenone	1101	2.99
42	1-terpineol	1115	0.95
43	Camphor	1123	3.40
44	Sabiena ketone	1133	1.15
45	Pinocarvone	1139	0.16
46	Unknown	1143	1.22
47	4-terpineol	1155	3.89
48	P-cymen-8-ol	1161	0.36
49	α-terpineol	1167	2.97
50	Unknown	1171	0.55
51	Trans-piperitol	1181	0.49
52	Trans-carveol	1191	0.18
53	Carvone	1217	0.37
54	Geraniol	1220	0.28
55	Chavicol	1225	0.26
56	Piperitone	1227	0.20
57	P-mentha-1, 8-dien-3-one	1243	0.15
58	Bornyl acetate	1255	0.23
59	Cuminic alcohol	1259	0.17
60	Carvacrol	1269	0.34
61	5, 5-dimethyl-1-Ethyl-1, 3-cyclopentadiene	1290	0.57
62	Eugenol	1327	2.75
63	Geranial formate	1345	2.03
64	Trans-methyl cinnamate	1354	1.02
65	Unknown	1362	0.88
66	Methyleugenol	1371	6.44
67	Cis-jasmone	1373	1.37
68	Trans-caryophyllene	1397	0.30
69	Unknown	1438	0.86
70	3-methoxy-2, 4, 6-trimethylphenol	1486	1.55
71	Phenyl ethyl tiglate	1553	0.66
72	Spathulenol	1558	2.00
73	Caryophyllene oxide	1563	0.82
74	Methyl-jasmonate	1613	0.42
75	2, 6-dimethyl-2, 6- octadiene-1, 8-diol diacetate	1681	1.46
76	Hexahydrofarnesyl acetone	1796	0.07

Table 3. Chemical composition of essential oils from *A. santolina* and *A. sieberi* by chemical class

Plant sample	*A. santolina*	*A. sieberi*
Chemical class	**%**	**%**
Monoterpene hydrocarbons	4.35	3.66
Oxygenated monoterpenes	68.22	69.22
Sesquiterpenes hydrocarbons	2.28	0.30
Oxygenated sesquiterpenes	1.50	2.82
Other hydrocarbons	1.13	2.49
Other oxygenated	17.68	18
Unknown	4.84	3.51
Terpenoid hydrocarbons	6.63	3.96
Oxygenated terpenoid	69.72	72.04
Total terpenoid	76.35	76.02
Total	100	100

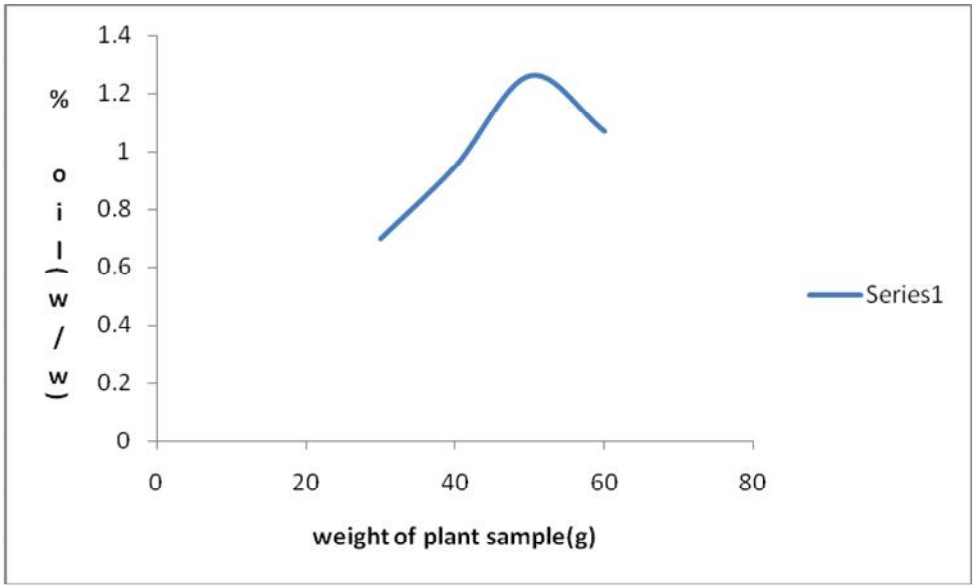

Figure 1. The weight optimization of the plant sample for extraction of essential oil from *A. sieberi*

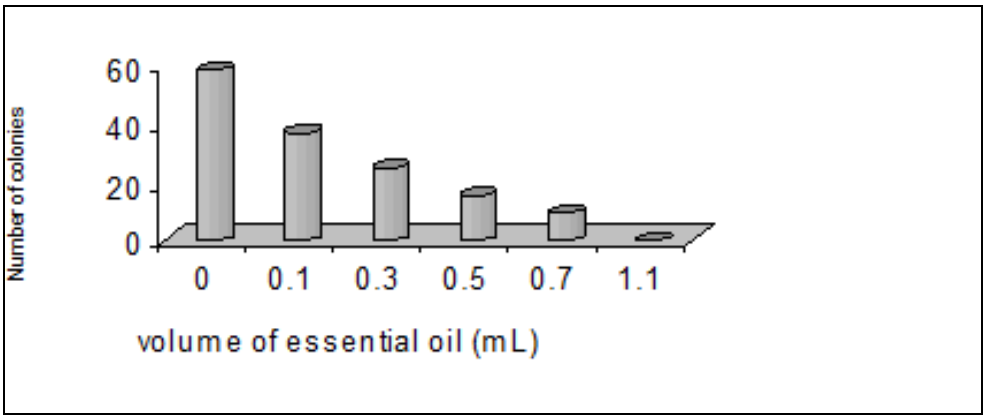

Figure 2. The variation of colony number as a function of the volume of essential oil from *A. sieberi*

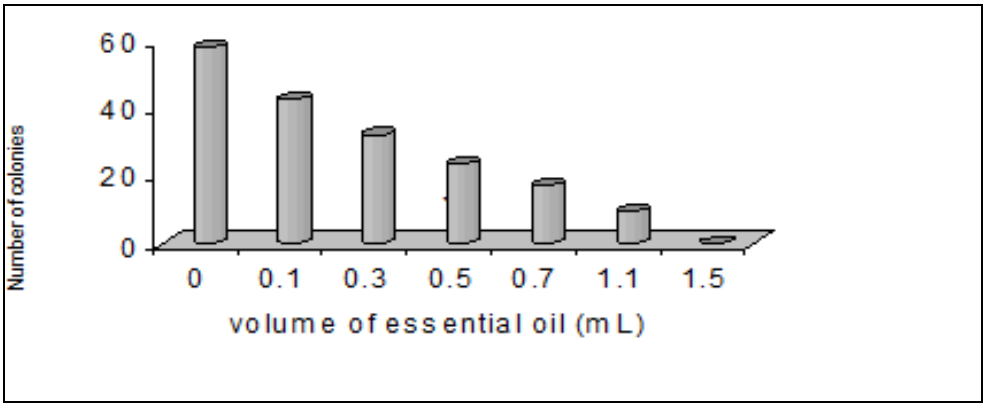

Figure 3. The variation of colony number as a function of the volume of essential oil from *A. santolina*