

Original Research Paper

# Antileishmanial and Antibacterial Activity of Essential Oils of Medicinal Plant *Achillea santolina* L.

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**Abstract:** *Achillea santolina* is medicinal plant widely used in folk medicine for gastrointestinal disorders, anti-inflammatory and anti-diuretic. The World Health Organization (WHO) reports that about 350 million people are considered to be at risk of contracting leishmaniasis especially among people living in the developing countries. This research will be the first time that evaluate antileishmanial activity of essential oils of *A. santolina* before and after flowering. Wild plants of *Achillea santolina* were collected from the Kalamoon Mountains in Syria before and after flowering. Essential oil of wild plants was identified by GC/MS analysis. The activity of essential oil was evaluated against five bacterial strains by well diffusion method and Promastigote lysis percentage was determined by an MTT assay. Chemical composition of essential oils of *A. santolina* was variable. According to our results the samples from Assal Al-ward and Deir Atiyah after flowering gave light yellow oils and the main constituents were Borneol 17.34% and Camphor 27.69% respectively. While the sample before flowering from Assal Al-ward gave blue oil due to presence Azulene 0.69% which absence in oils after flowering and the main component was 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) 13.05%. The essential oil of *A. santolina* at concentration 30% after flowering from Deir Atyah showed the highest inhibitory effect on *Klebsiella pneumonia* with diameter 21 mm. Also essential oil of *A. santolina* before flowering gave the highest antileishmanial activity with an IC<sub>50</sub> value (56.17 µg/mL). The antileishmanial activity of oils from *Achillea santolina* before flowering was better than after flowering, in contrast the antibacterial activity of oils before flowering was less than after flowering. Depending on the results, the oils from plants before flowering It could be constitute a potential treatment in the future.

**Keywords:** *Achillea santolina*, Antileishmanial, Antibacterial, Assal Al-ward, Deir Atiyah, Azulene

## Introduction

Increased interest in products of medicinal plants has been observed within recent years, due to the cosmetics, natural antimicrobial agents, pharmaceutical and foodpreservation systems. Leishmaniasis is a major public health problem especially in the developing countries. Leishmaniasis is recognized as one of the most neglected tropical diseases (Barrett and Croft, 2012). Visceral leishmaniasis is a disease caused by the *Leishmania infantum* in Europe, northern Africa and Latin America and *Leishmania donovani* complex in East Africa and the Indian subcontinent (Lukes *et al.*, 2007).

Anthroponotic cutaneous leishmaniasis is caused by *Leishmania tropica* and transmitted between humans by the *Phlebotomus sergenti* sand fly. Incidence in cutaneous leishmaniasis in Syria was 23,000 cases in year (Hayani *et al.*, 2015). In early 2013, an alarming increase to 41,000 cutaneous leishmaniasis cases was reported (Haddad *et al.*, 2015; Hayani *et al.*, 2015). The World Health Organization (WHO) reports that about 350 million people are considered to be at risk of contracting leishmaniasis especially among people living in the developing countries (WHO, 2010). The antileishmania and antimicrobial properties of plants have been investigated by a number of researchers around the

world, this is due to compounds synthesized in the secondary metabolism of the plant like the essential oils. There are continued studies for searching on treatments, especially search for new and better compounds that were produced of plant origin, because they are easily obtained at low cost. According to the World Health Organization about 80% of the people around the world turn to traditional medicine for their health care (Avijit *et al.*, 2007).

Latin name of Achillea comes from the name of Achilles, who used Achillea herb to healed the wounds at the time of the Trojan War, It was immortalized in the Iliad by Homer (Nowak *et al.*, 2010), in Arabic it is known as Qaysoom. Species name *santolina* means Sacred. Achillea is one of the youngest genera of the Asteraceae family, which is present throughout the world (Farajpour *et al.*, 2012). More than 100 species have been recognized in this genus (Goli *et al.*, 2008). *Achillea santolina* L. grows as a common herbal plant (perennial) in the north western Mediterranean region, this plant usually grows in barley and fallow fields, olive and figure plantations and at the edges of the small canals and roads (Darier and Tammam, 2012). These plants are native to Europe and western Asia but are also found in Australia, New Zealand and North America (Rechinger, 1963) and in some parts of Iran (Czygan, 2004). *A. santolina* L., is perennial herb, 10-30 cm long, with aromatic smell, the leaves are narrow, linear, pinnatisect, heads are small, yellow, 5-8 cm in diameter, arranged in small, flat inflorescence (Al-Eisawi, 1998). *Achillea santolina* L. Greyish woolly herb, stems branched, erect or ascending (Boulos, 2002). Flowering occurs during the spring season extending from March to May. Achillea has been used in medicine for its antihemorrhagic, healing and analgesic properties (Ody, 1993; Fleming, 2000). *A. santolina* is medicinal plant, widely used in folk medicine for gastrointestinal disorders (Bimbiraité *et al.*, 2008), its flower heads are used to relieve toothache, rheumatic pains and for treatment of colic and diabetes mellitus, also used as an anthelmintic and stomachic drug (El-Darier *et al.*, 2005). *A. santolina* has some traditional uses: Antiinflammatory, antidiuretic and antimicrobial effects (Al-Hindawi *et al.*, 1989; Twaij *et al.*, 1985; Cowan, 1999). This plant contains several polyphenols, a family of compounds with a great anti-diabetic potential and uses in Iraq, Egypt and Pakistan as antidiabetic, anti-inflammatory and to relieve pain. Also as vermicidal and carminative and also for stomach pain and hypertension (Alwwadi, 2013). *A. santolina* is dysentery and insect repellent (Khalil *et al.*, 2009). Eddouks and his colleagues had shown that *A. santolina* has some antimicrobial Activity and chemical analysis reported that *A. santolina* contains flavones, particularly flavonoids and polyphenols that have some beneficial antidiabetic effects (Eddouks *et al.*, 2003). Khalil *et al.* (2009) studied antimicrobial activity of Achillea santolina exerted against *Staphylococcus*

*aureus*, *Pseudomonas aeruginosa* and *Candida albicans* and showed that this medicinal plant can be used as natural antimicrobial agents in pharmaceutical and food preservation systems (Khalil *et al.*, 2009). Ardestani and Yazdanparast evaluated the effect of *Achillea santolina* extracts on lipid peroxidation, protein oxidation and antioxidant defense system such as Super Oxide Dismutase (SOD), Catalase (CAT) and reduced Glutathione (GSH) in the liver of Streptozotocin (STZ)-induced diabetic rats. Also they reported that the hydroalcoholic extracts of *Achillea santolina* reduced the blood glucose levels in rats (Ardestani and Yazdanparast, 2006). The essential oil of *A. santolina* contained 54 volatile components, the major components were 1, 8-cineole, fragranol, fragranyl acetate and terpin-4-ol (Si *et al.*, 2006; El-Shazly *et al.*, 2004). Motavalizadehkakhky *et al.* (2013) and his colleagues isolated and analysed the essential oils of *Achillea pachycephala* Rech.f. and *Achillea santolina* L. that obtained by steam distillation from the flowers, leaves and stems. The principle components In essential oils and extracts of *A. santolina* were 1, 8-cineole, camphor, terpinene-4-ol, fragranol, fragranyl acetate,  $\alpha$ -terpinyl acetate, caryophyllene oxide,  $\alpha$ -muurolol and some alkanes, alkanoic acids and esters. Oils and extracts showed higher activities against the tested gram negative bacterial strains (Motavalizadehkakhky *et al.*, 2013). No previous studies were found about antileishmania of *A. santolina*, so this research will be the first time that evaluate antileishmanial, so the aim of this study was to analysis essential oils of *A. santolina* before and after flowering and evaluation antibacterial and antileishmanial activity.

## Materials and Methods

### Materials and Extraction of Essential Oil

Wild plants of *Achillea santolina* were collected from the Kalamoon Mountains in Syria, from two sites. Before and after flowering from Assal Al-ward and after flowering from Deir Atiyah. Voucher specimens of the plants were deposited in the Department of Plant Biology, Faculty of sciences, Damascus University. The collected air parts were washed with clean water to remove soil, dried in room temperature and powdered using grindery. For production of essential oil, 100 g of air dried plant material was hydrodistilled for 3 h using a Clevenger apparatus. The oil was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and then was kept in a sealed vial at 4°C.

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was performed in Atomic Energy Commission in Syria (AECS) using a Variane 3400 equipped with a HB-5MS column (30×0.25 mm internal diameter, film thickness 0.25  $\mu\text{m}$  and with helium as a carrier gas) with analytic conditions: the injector and detector temperatures were held at 260°C.

The oven temperature program at first was 60°C for 4 minutes and then it was increased to 200°C and it remained at 200°C for 8 minutes. The final temperature was 260°C for 7.5 min. The SFE samples (1 µL) were injected using split mode with a split ratio of 1:40 and carrier gas was helium with split flow of 1ml/min. The ionization energy was 69.922eV with a scan time of 1 second and mass range of 35-450 amu.

#### Evaluation of Antibacterial Activity

The examined bacteria were obtained from Atomic Energy Commission in Syria (AECS). The bacteria include strains Gram positive: *Staphylococcus aureus* and Gram negative: *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. The density of each test bacterium suspension was set equalizes to 0.5 McFarland stander, which is 10<sup>8</sup> CFU/ml. Antibacterial activities were evaluated using well diffusion method (wells with diameter = 6mm) on Mueller-Hinton Agar (MHA). Wells were filled with 50 µl with different concentration of essential oils in DMSO (10, 20, 30%) and incubated at 37°C for 24 h. After the incubation period, the diameter of the growth inhibition zones was measured. The DMSO was used as negative standards. All tests were performed in triplicate.

#### Parasite Culture

The examined *Leishmania tropica* promastigotes were obtained from Faculty of Pharmacy, Damascus University. *L. tropica* promastigotes were cultured in RPMI-1640 liquid medium and supplemented with penicillin (100 UI/mL), streptomycin (100 µg/mL) and 5% Fetal Bovine Serum (FBS) at 26°C for preparation of adequate promastigotes. *L. tropica* promastigotes (10<sup>6</sup> parasites/ml) were incubated at 26°C for 24, 48 and 72 h in fresh RPMI-1640 medium.

#### Viability Assay

Promastigote lysis percentage was determined by an MTT [3-(4, 5-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay as described (Ebrahimisadr *et al.*, 2013), the bioassays were performed in triplicate. In 96-well microtitre plates, 3×10<sup>6</sup> promastigotes of *Leishmania tropica* per well were cultured in RPMI 1640 medium in the presence of essential oils in concentration of 25, 50, 75, 100 and 125 µg/mL and allowed to multiply for 24 h in the medium. After these times 20 µL of MTT (5 mg/mL) was added to each well and incubated in 18°C for 4 h then centrifuged in 1000g for 10 min, the supernatant was discarded and 100 µL of DMSO was added to each wells and re-suspended. The OD was read by ELISA reader in 540 nm:

$$\text{Viability \%} = (\text{Absorbance of Sample}/\text{Absorbance of Control}) \times 100$$

Results were expressed as the minimum concentration necessary for inhibition of 50% of parasite growth (IC<sub>50</sub>). The IC<sub>50</sub> values were calculated using Excel program.

#### Statistical Analysis

The data were statistically analyzed by one-way ANOVA (SPSS), followed by Tukey's multiple comparison test.

## Results

#### Components of Essential Oils

The hydrodistillation of the aerial parts of *Achillea santolina* samples that were collected from two different locations, the samples after flowering (A1: Assal Al-ward and A2: Deir Atiyah) gave light yellow oils, while the sample before flowering (A3: Assal Al-ward) gave blue oil due to presence Azulene 0.69% which absence in oils after flowering. The general chemical profiles of the tested oils, retention time and the percentage content of the individual compounds with amounts (>1%) except the component Azulene are summarized in Table 1. The analysis showed the main components of the sample from Assal Al-ward after flowering were Borneol 17.34%, 3-Cyclohexen-1-one, 2-isopropyl-5-methyl 9.91%, Eucalyptol (1,8-cineole) 9.66%, 2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl) 7.34%, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) 6.42%, 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) 5.15%, Terpineol, cis.-beta 3.72%. The second sample from Deir Atiyah after flowering showed different proportions and components of their main constituents, the main components were characterized by large amounts of Camphor 27.69%, Eucalyptol 11.71%, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) 8.01%, 3-Cyclohexen-1-one, 2-isopropyl-5-methyl 6.36%, 2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl) 3.54%, Camphene 3.43%, 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) 3.29%. In compare with the third sample from Assal Al-ward before flowering the main components were 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) 13.05%, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) 12.08%, Eucalyptol 9.6%, 2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl) 9.14%, Terpineol, cis.-beta 6.29%, 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl) 4.78%, 1,6-Dimethylhepta-1,3,5-triene 3.16%. Other components were presented in amounts less than 3% (Table 1).

#### Antibacterial Activity

The results showed that concentration 30% has more activity than lower concentrations 20 and 10% in each tested bacteria Table 2. In this concentration 30% the essential oil of *A. santolina* after flowering from Deir

Atyiah showed the highest inhibitory effect on *Klebsiella pneumonia* with diameter 21 mm and the essential oil of *A. santolina* before flowering from Assal Al-ward showed the lowest effect on *Escherichia coli* and

*Salmonella typhi* with diameter 12 mm. In general essential oils from Deir Atyiah after flowering was more affective at all concentrations. *Pseudomonas aeruginosa* was not sensitive to all tested essential oil.

**Table 1:** Chemical composition of essential oils of wild plants *Achillea santolina*

	RT	A1	A2	A3
Camphene				
Compounds	9.32	-	3.43	-
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)	10.97	-	1.51	1.68
Cyclohexene, 1-methyl-4-(1-methylethylidene)	13.88	1.03	1.5	2.91
Eucalyptol (1,8-cineole)	16.06	9.66	11.71	9.6
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	17.58	2.1	-	4.78
Terpineol, cis-.beta.-, Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	19.41	1.83	1.77	2.69
Cyclohexene, 1-methyl-4-(1-methylethylidene)-, Carene	19.82	-	-	1.19
Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)	22.67	1.98	1.51	-
Bicyclo[3.1.0]hexan-3-one,4-methyl-1-(1-methylethyl)-,[1S-(1.alpha.,4.beta.,5.alpha.)]	24.31	-	-	1.46
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)	24.76	5.15	3.29	13.05
Terpineol, cis-.beta.	27.06	3.72	2.46	6.29
1,6-Dimethylhepta-1,3,5-triene	27.8	-	-	3.16
Silane, trimethyl(4-methyl-3-pente n-1-yanyl)-	28.43	-	-	1.06
Borneol	29.89	17.34	-	-
Camphor	29.97	-	27.69	-
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	30.37	6.42	8.01	12.08
p-menth-1-en-8-ol	32.18	1.35	1.34	2.14
2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)	33.57	7.34	3.54	9.14
Benzene, 2-ethyl-1,4-dimethyl-	36.56	-	-	1.09
Pulegone	38.42	1.86	-	-
3-Cyclohexen-1-one, 2-isopropyl-5-methyl-	40.86	9.91	6.36	1.59
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate	44.72	-	-	1.39
Caryophyllene	47.33	-	-	1.48
3,5-Heptadien 2-ethylidene-6-methyl	53.02	1.02	-	2.87
Caryophyllene oxide	64.78	1.27	1.38	-
10,10-Dimethyl-2,6-dimethylenebicy clo[7.2.0]undecan-5.beta.-ol	69.42	2.04	2.22	-
Azulene	77.84	-	-	0.69

After flowering (A1: Assal Al-ward, A2: Deir Atyah). Before flowering (A3: Assal Al-ward)

**Table 2:** Antibacterial activity of essential oils of wild plants *Achillea santolina*

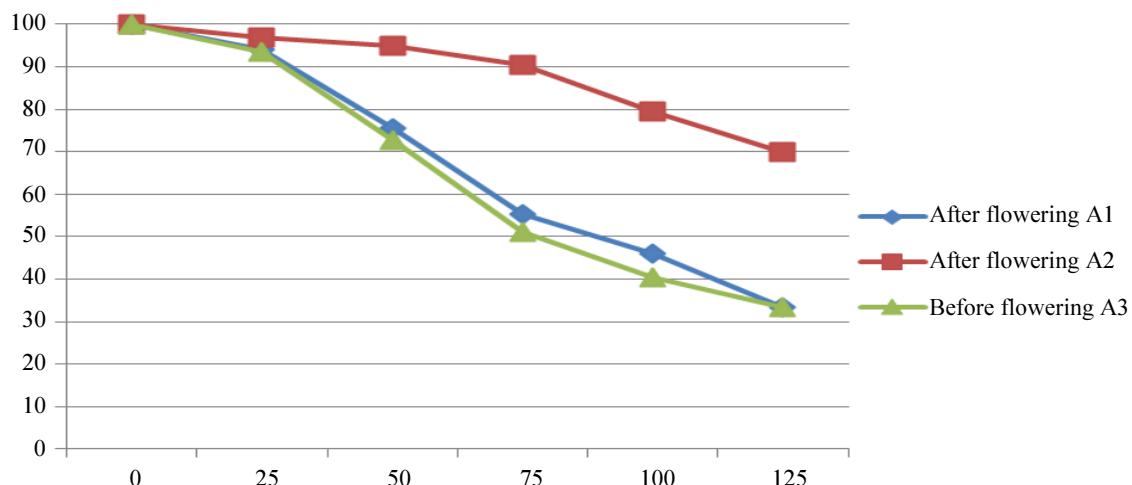
Tested Bacteria	Source of plants and concentration of essential oils and diameter of inhibition zone with mm								
	10%			20%			30%		
	A1	A2	A3	A1	A2	A3	A1	A2	A3
<i>Salmonella typhi</i>	10±0.1	na	na	13.5±0.5	10.1±0.1	8±0.7	17±0.3	13±0.2	12±0.9
<i>Staphylococcus aureus</i>	8.6±0.09	12.2±0.2	na	13±0.2	14.3±0.2	9±0.5	16±0.4	18±0.1	13±0.1
<i>Klebsiella pneumonia</i>	na	14.9±0.3	na	10±0.1	18.1±0.9	12.4±0.5	14±0.7	21±0.5	15±0.3
<i>Escherichia coli</i>	na	12.5±0.2	na	11±0.0	15.1±0.4	8±0.2	15±0.1	18±0.7	12±0.06
<i>Pseudomonas aeruginosa</i>	na	na	na	na	na	na	na	na	na

na: not active, ±: Standard deviation.

After flowering (A1: Assal Al-ward, A2: Deir Atyah). Before flowering (A3: Assal Al-ward)

**Table 3:** Antileishmanial assay of essential oils of wild plants *Achillea santolina* after 24 h of treatment

Source of plants	Concentration of essential oils (µg/mL) and Viability%±SD						
	control	25	50	75	100	125	IC <sub>50</sub>
Assal Al-ward A1	100±0.0	94±0.3	75.6±0.4	55.2±1.3	46.1±0.1	33.5±0.3	60.84±0.1
Deir Atyah A2	100±0.0	97±0.7	94.8±0.5	90.3±0.9	79.4±0.7	70±0.5	87.15±0.2
Assal Al-ward A3	100±0.0	93.4±1.1	72.7±0.2	51±0.7	40.5±0.9	33.3±0.5	56.17±0.5



**Fig. 1:** Activity of essential oils of wild plants *Achillea santolina* against leishmania after 24 h of treatment

#### Anti-Leishmanial Activity of Essential Oils

Growth inhibition of *Leishmania tropica* promastigotes was evaluated and the percentage of viability in the presence of various concentrations of essential oils of *Achillea santolina* in comparison with control for 24 h are showed in Table 3 and Fig. 1. Our findings showed that essential oils of *Achillea santolina* inhibited promastigote growth with percentage of viability ranged from 33.3% to 97%. With a concentration increase of essential oils the optical density significantly decreased and inhibitory percentage increased. The best activity of essential oils was of *A. santolina* from Assal Al-ward before and after flowering with viability 33.3 and 33.5% respectively at concentration 125 µg/mL. Essential oil of *A. santolina* before flowering gave the highest antileishmanial activity with an IC<sub>50</sub> value (56.17 µg/mL) and was more active compared with essential oils of *A. santolina* after flowering from Assal Al-ward and Deir Atiyah with IC<sub>50</sub> value (60.84 and 87.15 µg/mL), respectively.

## Discussion

#### Components of Essential Oils

The aim of the present study was to determine the chemical composition of the essential oils of *A. santolina* from different locations before and after flowering from Kalamoon Mountains in Syria. Chemical composition of essential oils of *A. santolina* was variable. According to our data, Borneol 17.34%, Camphor 27.69% and 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) 13.05% were the main constituents of *A. santolina* from Assal Al-ward and Deir Atiyah after flowering and Assal Al-ward before flowering, respectively. Which may be due to the differences in environmental conditions and date of collection of plants before and after flowering.

Previous studies that have determined the chemical composition of *A. santolina* essential oils from Iran showed also identified high levels of Fragrantyl acetate 28.4%, 34% and 37% from flowers, leaves and steam, respectively (Motavalizadehkakhky *et al.*, 2013). Our results didn't show differences with percentage of eucalyptol before flowering (9.6%) and after flowering (9.66%) from Assal Al-ward, but there is clear increasing with percentage of camphor from <1% to (17.34%), these findings confirmed that essential oil composition of plant can be different in quality and quantities in different period of growth of plant, that is disagree with another study (Al-Jaber *et al.*, 2016) where *A. santolina* was collected at different growth stages, eucalyptol was the main constituent at the pre-flowering (18.05%) and increase compare with flowering (20.51%) and camphor decrease from (9.51%) before flowering to (7.66%) after flowering. There are many similarities between the oils although the amounts of some corresponding compounds are different. Our results agree with (Dastjerdi and Mazoji, 2015) suggested that these differences may be related to the different geographical origins of the samples. In general, the differences in oil composition may be due to , plant genetic type, different environmental factors, seasonality, physiological age and developmental stage.

#### Antibacterial Activity

Antibacterial properties due to many components like Eucalyptol (1,8-cineole) and 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) which were found with high percentage in all tested essential oils. Eucalyptol is one of the most important compounds as antibacterial activity (Leung and Foster, 2003). 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) which is derivative of limonene (Olivera *et al.*, 2001) and Limonene has been implicated

to be an effective antimicrobial agent (Magwa *et al.*, 2006). Our results showed that *Klebsiella pneumonia* was the most sensitive bacteria to the plant oils from Deir Atiyah (21 mm). It is revealed that the *Klebsiella pneumonia* was sensitive to the essential oil of *A. santolina* from Iran with inhibition zone from 21.5 to 23 mm (Motavalizadehkakhky *et al.*, 2013). The activity of oils from Deir Atiyah may be due to presence high amount of Camphor 27.69% that has the most efficient antibacterial property (Prudent *et al.*, 1993; Aliannis *et al.*, 2000). All of oils didn't show any effect against *P. aeruginosa*, that is disagree with result of Khalil and his colleagues where reported that extracts from *Achillea santolina* inhibited *P. aeruginosa*, with the high effect (Khalil *et al.*, 2009) that is because they used ethanol extracts.

#### *Anti-Leishmanial Activity of Essential Oils*

Our results showed that the differential antileishmanial activity of these oils against the promastigotes is related to the differential composition of such oils depending on sources of plants and stage of growth. No previous study or reports about effect of *A. santolina* as antileishmanial, so we suggested the active components in oils especially *A. santolina* from Assal Al-ward before flowering can interact with mitochondrial membranes leading to its death by apoptosis. Santos and his colleagues tested essential oil activity of *Achillea millefolium* against *Leishmania amazonensis* and IC<sub>50</sub> was 7.8 µg/ml, they reported that Azulene has antileishmania activity (Santos *et al.*, 2010), their result supports our findings because the highest activity was in oil that contain Azulene before flowering. Caryophyllene also was found in *A. santolina* oil before flowering and has antileishmanial activity recording to Soares and his colleagues, their studies pointed out Caryophyllene in *Copaifera* spp. oil was an effective antileishmanial compound against *L. amazonensis* (Soares *et al.*, 2013). Also Chouhan and his colleagues reported that Hexane and Ethanolic extracts of *Piper nigrum* which contain Caryophyllene inhibited the growth of *Leishmania donovani* promastigotes with IC 5031.6 and 37.8 µg/ml, respectively (Chouhan *et al.*, 2014). Another study showed the antileishmanial activity of Caryophyllene was at least two times more potent than limonene against *L. amazonensis* with IC 50 value 96 µM (Moura *et al.*, 2012). Another compounds like Eucalyptol and Borneal did not have antileishmanial activity according to Machado and his colleagues, they did not showed any biological activity when tested extracted essential oil from *Thymus capitellatus* against *Leishmania infantum*, *Leishmania tropica* and *Leishmania major* promastigotes at the tested concentrations (Machado *et al.*, 2014).

## Conclusion

Essential oils of *Achillea santolina* before and after flowering showed clear different with color, chemical components and activity. The antileishmanial activity of oils from plants before flowering was better than after flowering, in contrast the antibacterial activity of oils before flowering was less than after flowering. Also the antileishmanial activity of oils was more than antibacterial activity. Depending on the results, the oils from plants before flowering It could be constitute a potential treatment in the future.

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## Authors' Contributions

**Abdulkarim Dakah:** Design and work of the experiments, analyzed and interpreted data, wrote the manuscript.

**Mohammed Maarrouf:** Contributions to design experiments, analysis and interpretation of data.

## Ethics

This article is original and it is the result of the author's research, so there are no ethical issues.

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