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Assessment the Efficacy of Some Natural Herbal Plants in the Treatment of Acne Vulgaris

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Abstract:

Background Acne vulgaris is considered one of the most prevalent skin disorders that affect mainly young adults who usually need a safe, affordable and effective treatment options that can manage their condition for a long time with minimum side effects.

The aim of this work was to clinically test the efficacy of using some herbal plants after testing its cytotoxicity and anti-inflammatory ability in controlling acne vulgaris.

Methods This multistep study started by choosing some essential oils that waere previously classified as anti-inflammatory agents, we analysed their chemical components and tested them for cytotoxicity and anti-inflammatory effect on row cells and human fibroblasts to choose the best ones to formulate a cleanser and gel that were tested on seven patients of acne vulgaris,

Results There was a very good improvement in the acne lesions as regard the count and inflammation after applying the cleanser and the gel formulated from the essential oils that were proved to be safe and effective.

Conclusion There is a strong evidence that combination of essential oils like lavender oil, chamomile oil, tea tree oil and Juniperus Communis Fruit oil can be used safely in the treatment of acne vulgaris.

Keywords: Acne vulgaris, essential oils

1. Introduction

Acne vulgaris is a very common skin disorder that affects young people at an important period in their lives and they continue to suffer from it for many years. In the meantime, they can follow many treatment protocols that may help them for a short period or lead to long term remission but with many unpreferable side effects [1].

Acne vulgaris is considered a multifactorial disorder and its pathogenesis is very complicated starting by an increase in sebum production and this is followed by inflammation of variable degrees and keratinocyte proliferation and also bacterial colonization [2].

Many treatment options are involved in the treatment of acne vulgaris aiming to decrease the inflammation or the bacterial activity and to regulate the sebum production. However, repeated use of antibiotics may lead to bacterial resistance and decrease the efficacy of the treatment that ends to repeated exacerbations of the disease. Also, resorting to the use of retenoids as a final solution to eliminate the disease usually accompanied by many side effects that may be intolerable for the patients [3].

There is an increasing interest world widely for the use of the complementary medicines especially for diseases that require long periods of treatment. Essential oils, like tea tree oil and lavender oil, are considered the commonest alternative medicines that can be used to treat many cutaneous disorders as they have strong antimicrobial ability [4].

When we make a search about acne vulgaris, we can find many trials that used herbal products to decrease the symptoms of the disease as the previous use of juniper berry oil based on what is known about its ability to regulate the sebum production and its antibacterial effect [5 &6].

In this study, we evaluated the ability of different herbal essential oils blend with aloe vera, which is known by its anti-inflammatory effect, in the treatment of inflammation on raw cells and then we followed that by their application on human skin fibroblast to confirm its efficacy and safety before choosing the best ones among them to be formulated in the form of gel and fascial cleanser to be applied on the skin of human patients suffering from acne.

2. MATERIALS AND METHOD

2.1 Materials:

Chemicals: Carbapol 940, cremophore co 40, guar hydroxypropyltrimonium chloride, triethanolamine, Ethylene glycol2-monophenyl ether-octoxyglycerin, sodium laureth sulfate and cocamidopropylbetaine were purchased from Pharmalog chemical company, Alexendria.

Essential oils and herbal extract: Lavender oil LVO (Lavandula *angustifolia) and* chamomile essential oil (CO) were purchased from the unit of pressing and extracting natural oils of National Research centre, Cairo. Tea tree oil *Melaleuca alternifolia)* TTO and *Juniperus Communis Fruit oil* (JO) were purchased from Harraz company, Cairo. Aloe barbadensis leaf extract and Bisapolol were purchased from Pharmalog chemical company.

Table 1 showed Chemical composition of chamomile, lavender, juniper, and tea tree essential oils

Gas chromatography-mass spectrometry analysis (GC-MS analysis)

The GC-Ms analysis was carried out according to Said-Al Ahl *et al*. [7] and Omer *et al*. [8], using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications.

Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 60 ^{o}C for 1 min; rising at 3.0 ^{o}C /min to 240 ^{o}C and held for 1 min. The injector and detector were held at 240 ^{o}C. Diluted samples (1:100 hexane, v/v) of 1 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

2.2 Herbal formulations:

For preparation of herbal formulations, gel and facial cleanser were prepared. The gel was prepared using carbapol 940 (0.5%) w/v that was dispersed in distilled water with stirring on hot plate at temperature not more than 60°c, to form gel aspect. Aloe barbadensis leaf extract (10%) v/v, essential oils blend and cremophore co 40 were added according to tables shown below. The dispersion obtained was neutralized with required quantity of triethanolamine to obtain pH 5 to 5.5. Ethylene glycol2-monophenyl ether-octoxyglycerin was added with 1% v/v and the formulations then put inside a suitable container and labelled accordingly.

Cleanser was manufactored according to Solanki *et al* [9] by weighting 0(0.5-1) % w/v guar hydroxypropyltrimonium chloride and dispersing it in hot distilled water (half the batch) not more than 60°C with moderate stirring. In the remaining amount of water add the primary surfactant (sodium laureth sulfate) % v/v, then secondary surfactant (cocamidopropylbetaine) (4%) then finally Ethylene glycol2-monophenyl ether-octoxyglycerin was added with 1% v/v. Finally herbal essential oils (TTO, JO) were added with (1, 0.4) %v/v respectively.

2.3 Measurement of particle size and zeta potential

Samples were sonicated for 2 minutes then diluted 100X with deionized water before measurement.

The prepared particles were analyzed for their particle size and size distribution in terms of the average volume diameters and polydispersity index by photon correlation spectroscopy using particle size analyzer Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom) at fixed angle of 173° at 25° C. Samples were analyzed in triplicate. The same equipment was used to determine zeta potential.

2.4 Cytotoxicity tests on raw cells

1. Cell culture

RAW 264.7: Mouse macrophage cell line was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO2atmosphere at 37 °C.

2. Cytotoxicity assay

Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension (5x10^3 cells) were in 96-well plates and incubated in complete media for 24 h. Cells were left within another aliquot of 100 µL media possessing drugs of different concentrations. Then after 72 h of exposure to the drud, cells were fixed by changing the media with 10% TCA OF 150 µL and LEFT at 4 °C for

only an hour. After that the TCA solution was removed, and the cells were washed 5 times using distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added to the cells and incubated in a dark room of natural temperature for 10 min. 1% acetic acid was used to wash the plates for 3 times before leaving them to dry by air overnight. Then, 150 µL of TRIS (10 mM) was added to help the dissolving of the protein-bound SRB stain; A BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany) was used to measure the absorbance at 540 nm [10&11].

2.5 anti-inflammatory tests on raw cells

1. Cell culture

The RAW 264.7 cells: Mouse macrophage cell line was taken from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were kept within DMEM media containing 100 units/mL of penicillin, 100 mg/mL of streptomycin in addition to 10% of fetal bovine serum (inactivated by heating) in humidified atmosphere with 5% (v/v) CO2 and at a temperature of 37 °C.

2.In-Vitro Anti-inflammatory assay

RAW264.7 Cells were seeded into a 96-well plate and incubated for twenty-four hours. The next day, inflammation was induced with 1 µg/mL of LPS (LPS-group) and untreated cells will be replenished with fresh media (Control group). Compounds will be treated with LPS in two/five concentrations (LPS+ Drug). Dexamethasone (1uM) was included as an antiinflammatory positive control. To measure nitric oxide (NO) secretion, equal volumes of the cell supernatant and Griess reagent were mixed for 10 min in the dark place at room temperature. The absorbance at 540 nm representing the nitrite concentration was estimated using the ELISA plate reader [12 &13].

2.6 Safety tests on Human skin fibroblast (HSF)

1.Cell culture

HSF: Human Skin Fibroblast was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were stored in DMEM media containing the same supplement and in the same conditions mentioned above with the row cells.

2.Cytotoxicity assay

Cell viability was assessed by SRB assay by the same steps followed in the validity assessment of the row cells.

2.7 application of the formulation on acne patients:

Seven patients with mild to moderate acne vulgaris from the dermatology outpatient clinic in the National Research Centre agreed to participate in this study. Patients with severe acne or taking any systemic medication for the last 3 months or using any topical medications in the previous two weeks were excluded from the study. After the patients signed the written consents, they were given the formulated cleanser and gel to be used at home according to the following instruction:

1- The patients would put the gel over the acne lesions for half an hour.
2- Then they were asked to wash the gel by using the cleanser that should be applied to the whole face
3- These steps had to be repeated every night.
4- Patients were asked to avoid direct sun exposure during the period of using the formulation.

After three weeks we reexamined the patients to re-evaluate the acne lesions and to report any side effects observed.

Assessment of the acne lesions was done by using standard photos and also, we used the counting method to count the number of the inflammatory acne lesions in the whole face.

Statistical analysis then carried out simply by using SPSS (statistical program for social science version 23) to describe quantitative variables that were expressed as Mean, SD, Median and IQR according to shapiro test of normality. And to compare quantitative variables between the two groups in non-parametric data (SD>30% mean) by using Mann Whitney test.

P value >0.05 insignificant P<0.05 significant

3. RESULTS

GC-MS analysis of the chemical composition of chamomile, lavender, juniper, and tea tree essential oils resulted in identifying 60 compounds. All identified compounds and their percentage composition were given in Table 1. Twenty-four compounds were identified in chamomile essential oil, most of them were oxygenated sesquiterpenes comprising about 57.2 %; followed by sesquiterpene hydrocarbons with 35.4 %. Monoterpene hydrocarbons consist less than 1% of the identified compounds. Based on presented results, it can be concluded that α-bisabolol oxide A is the most abundant component (47.6), followed by *trans-β*-farnesene (31.3 %). Lavander essential oil analysis resulted in the identification of 18 compounds, which were mainly oxygenated monoterpenes (97.2%). The highest component percentage was recorded to linalyl acetate (57.1%), followed by α-linalool (29.1%). Twenty-two compounds were detected in juniper essential oil. Monoterpene hydrocarbons represent the majority with percentage up to 96.5%. α-Pinene is the most abundant component (77.2%), followed by limonene (15.3%). Eighteen compounds were determined in tea tree essential oil, about half of them is monoterpene hydrocarbons (48.7%), while the other half is oxygenated monoterpenes (48.6%). The major component is 4- terpineol comprising (40.8%), followed by γ-terpinene (21.9%) and *p*-cymene (13.5%). Monoterpenes are abundant in lavender, juniper, and tea tree oils, while sesquiterpenes are major in chamomile oil.

Table (1): Chemical composition of chamomile, lavender, juniper, and tea tree essential oils

Compound name	RI	Chamomile oil		Lavander oil		Juniper essential oil		Tea tree essential oil	
		Rt	Peak area %	Rt	Peak area %	Rt	Peak area %	Rt	Peak area %
Monoterpene hydrocarbons									
α-Pinene	939			7.75	0.16	7.77	**77.19**	7.76	4.62
Camphene	953					8.16	0.23		
β-pinene	970					9.07	0.96		
α- Myrcene	986					9.53	1.43	9.54	0.15
α-Phellandrene	1000			9.06	0.01	8.92	0.27	10.04	0.15
4- Carene	1009							10.52	6.19
δ-3-Carene	1013	11.69	0.40			10.34	0.22		
P-Cymene	1027	10.60	0.28	10.62	0.51	10.62	0.81	10.63	**13.48**
Limonene	1028					11.01	**15.33**		
γ-Terpinene	1053	12.12	0.13			12.13	0.07	12.16	**21.87**
α- terpinene	1055					13.38	0.18		
p-Mentha-1,4(8)-diene	1261							13.40	2.27

cis-Verbenol	1148					15.52	0.06		
2-Carene	948			25.00	0.25				
Oxygenated monoterpene									
Eucalyptol	1031							10.94	3.10
Cineole	1032			10.95	2.8				
trans-Linalool oxide	1086							12.52	0.04
α-Linalool	1099			13.73	**29.08**			13.67	0.04
Camphor	1145			15.11	5.42	16.41	0.21		
Isoborneol	1155			16.41	0.02				
4- Terpineol	1181			17.84	0.11	17.00	0.16	17.09	**40.84**
α-Terpineol	1185			17.51	0.74			17.53	4.64
Isopulegol acetate	1210			19.8	0.25				
Linalyl acetate	1275			20.8	**57.1**				
Dihydro-carvyl acetate	1282			21.82	0.05				
Bornyl acetate	1290			21.98	1.54	21.94	0.36		
Lavandulyl acetate	1295			24.16	0.08				
ɣ -Muurolene	1469					28.15	0.30		
Sesquiterpene hydrocarbons									
(-)-Aristolene	1433							28.12	0.10
Isocaryophillene	1412					28.41	0.09		
Aromadendrene	1428							29.28	0.60
Humulene	1442					29.51	0.08		
trans-β-Farnesene	1452	29.93	**31.31**						
(+)-Ledene	1453							31.56	0.44
α-Chamigrene	1442					31.55	0.05		
α-Copaene	1434	30.89	1.80			31.68	0.05		
δ -Elemene	1436	31.53	1.07						
(E, E)-α-Farnesene	1516	31.97	0.97						
δ-Cadinene	1528	32.18	0.09						
12- Thujopsene	1528					32.23	0.26		
Cadina-1(10),4-diene	1529	32.65	0.18			32.57	0.10	35.58	0.16
Chamazulene	1732	39.75	1.27						
Oxygenated Sesquiterpene									
Caryophyllene oxide	1565	30.29	0.06						
Spathulenol	1579	34.40	0.10						
Ledol	1617							34.82	0.11
Cedrol	1625					35.35	1.00		
α-Cadinol	1646	36.83	0.80						
α-Bisabolol oxide B	1660	37.44	3.19						
Cis Lanceol	1699	38.16	4.13						
α-Bisabolol	1700	38.46	1.32						
α-Bisabolol oxide A	1747	40.54	**47.57**						
Others									
2-Methyl-ethylbutanoate	854	5.18	0.32						
2-Pentylfuran	994	9.38	0.04						
3-Methyl-2-cyclobutyl butanoate	1109	11.93	2.21						
2,7-Dimethyl-2,6-octadien-4-ol	1221	13.02	0.15						

2-Methyl-4-octenal	1299							18.05	0.11
2,4-Dimethyl-2,4-heptadienal	1365			21.66	0.88				
(E)-1-(2,3,6-trimethylphenyl) buta-1,3-diene	1550	31.79	0.19						
1-(Phenylethynyl)-1-cyclohexanol	1801	44.21	1.87						
Monoterpene hydrocarbons			0.81		0.93		**96.45**		**48.77**
Oxygenated monoterpene			0		**97.19**		1.03		**48.62**
Sesquiterpene hydrocarbons			**36.69**		0		0.93		1.3
Oxygenated Sesquiterpene			**57.17**		0		1.0		0.11
Other compounds			4.78		0.88		0		0.11
Total identified compounds			99.45		99.0		99.41		98.81

Particle size analysis

Herbal gel containing LVO, TTO, JO with (13.33 mg/ml, 6,67 mg/ml, 6,67 mg/ml) respectively was analyzed for particle size analysis that was 2.2e4 ± 2.4e4 nm and PDI (a representation of the distribution of size population) 0.86 ± 0.23.

Cytotoxicity tests on row cells

LVO (R) with two concentrations: 2 mg/ml,5 mg/ml and herbal gel (B22) containing (LVO, TTO, JO) with two concentrations: (4.44, 2.22, 2.22) mg/ml and (3.33, 1.67, 1.67) mg/ml respectively and total concentrations (6.67 mg/ml, 8.89) mg/ml. were tested for their cytotoxicity on raw cells with the following results shown in table 2; Results showed that cell viability using LVO not exceed 75% viability of raw cells which is not recommended to enter anti-inflammatory experiments, on the contrary the herbal gel showed about 99% cell viability which is preferable to be tested on raw cells for its anti-inflammatory activity.

Table (2): Results of cytotoxicity tests on row cells:

		Inhibition %			Blank Corrected Data			Raw data			B22
STD	**Mean**	**3**	**2**	**1**	**3**	**2**	**1**	**3**	**2**	**1**	**conc**
0		0	0	0	0.131	0.13	0.141	0.2	0.199	0.21	C
1.53343632	12.9353	14.9254	12.6866	11.194	0.114	0.117	0.119	0.183	0.186	0.188	6.67 mg/ml
1.82797742	3.73134	5.97015	3.73134	1.49254	0.126	0.129	0.132	0.195	0.198	0.201	8.89 mg/ml
		0.134	Control average		0.069	Blank Average		0.069	0.069	0.069	Blank

Anti-inflammatory tests on row cells

Results showed that B22 with lower concentration of essential oils blend showed higher activity for inhibiting NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7.it was mainly due to anti-inflammatory activity of LVO in Aloe Barbadensis gel form with TTO and JO.

Then, different herbal blends were prepapred with different EOS tht were known for their anti-inflammatory activity. Here LVO (commonly used as an anti-inflammatory EO in the first blend was substituted by CO in the second blend and by Bisapolol in the third one according to table 3:

Table (3): Results of anti-inflammatory tests on row cells:

B24b mg/ml	B24a mg/ml	B23b mg/ml	B23a mg/ml	B22b mg/ml	B22a mg/ml	Blend name
16	8	19	9.5	29	14.5	**Total conc of oils blend**
7	3.5	7	3.5	7	3.5	**TTO**
7	3.5	7	3.5	7	3.5	**JO**
--	--	--	--	15	7.5	**LVO**
--	--	5	2.5	--	--	**CO**
2	1	--	--	--	--	**Bisapolol**
100	50	100	50	100	50	**Aloe Barbadensis**
820	410	809	404.5	751	375.5	**Water**
64	32	72	36	120	60	**Cremophore co 40**

Safety tests on Human skin fibroblast (HSF)

Herbal gel containing LVO B22 with total blends 14.5 and 29 mg/ml were tested ror their cytotoxicity on human skin fibroblast (HSF) and showed cell viability up to 94% that was saver than cytotoxicity tests on raw cells with the following results:

B22 (1hr)	Raw data			Blank Corrected Data			Viability %				
Conc	1	2	3	1	2	3	1	2	3	Mean	STD
c	6.214	6.142	6.055	6.11633	6.04433	5.95733	100	100	100	100	0
14.5mg/m	5.928	5.768	5.606	5.83033	5.67033	5.50833	96.5394	93.8901	91.2076	93.879	2.17667725
29mg/ml	5.362	5.417	5.409	5.26433	5.31933	5.31133	87.1675	88.0782	87.9457	87.7304	0.4017406
Blank	0.099	0.099	0.095	Blank Average		0.09767	Control average		6.03933		

Figure (1) results of safety tests on human fibroblasts

In vivo study on individuals under the supervision of the Department of Dermatology and Venereology Research at the National Research Center:

The gel containing (LVO, TTO, JO with concentrations 3.75,7,7 mg/ml respectively) and cleanser mentioned previously were used for seven patients suffering from both types of acne (inflammatory and non-inflammatory), and the results showed a noticeable improvement in both types in terms of reducing inflammation and pus-filled pimples and significant decrease in the count of acne lesions in just two weeks (without resorting to oral antibiotics or the use of isotretinoin that has possible side effects on the liver and externally, as it leads to dry skin.

Some pictures of patients before and two weeks after using the products, showing the effect of the products in reducing keratosis and getting rid of microbes and associated infections.

4. Discussion

Acne vulgaris is a highly prevalent cutaneous disorder among adolescents that lead to unfavourable appearance and psychological discomfort. Sebaceous hyperactivity, hyperkeratinisation, bacterial infiltration, and inflammation are the leading causes for the development of acne vulgaris [14]. Over the years, many acne treatment protocols have evolved targeting the factors involved in the pathogenesis of acne. Many recently developed antiacne medications have been supplemented with natural, safe, and effective agents such essential oils or their ingredients [15].

The mostly recommended essential oils for the treatment of acne should be characterized by having strong anti-inflammatory effect with calming and toning behaviour such as tea tree oil, lavender oil, thyme and lemon. Many studies have suggested different essential oils for the treatment of acne but there was a need for further evidence to fully prove their therapeutic ability [15].

In this study, we tested the ability of different herbal essential oils, known to have anti-inflammatory potential combined with aloe vera as a calming agent, on raw cells and then we followed that by their application on human skin fibroblast to confirm its efficacy and safety before choosing the best ones among them to be formulated in the form of gel and fascial cleanser to be applied on the skin of human patients suffering from acne.

On comparing our results of the Gc-MS analysis of chamomile essential oil with the previous reported chromatographic profiles, it revealed similarity in the abundance of oxygenated sesquiterpenes in the essential oils obtained from dried chamomile flowers and chamomile teabags with percentage (66.85-77.48%). The main component in the chamomile flower oil was α-bisabolol oxide A with percentage 57.2 %. The content of bisabolol has been established to be higher in direct sunlight dried flower heads of chamomile [16].

Another study showed similar *trans-β*-farnesene content (29.8%) but it showed some differences in chamazulene content, a bicyclic sesquiterpenes generated by distillation from matricine [17].

The analysis of lavender essential oil unveiled redundancy of oxygenated monoterpenes which are in line with the previous reports. A study adduced the prevalence of oxygenated monoterpenes (31.5 %) with two main components linalyl acetate (26.6%), followed by linalool (19.7%) [18]. Compared with literature data, the examined juniper essential oil showed higher α-pinene content than that reported of the oil from the berries of Estonian juniper (47.9%), Greek juniper (27 - 62%), Polish and French (45 – 80%), and Italian juniper (52.3%) [19]. The analysis of TTO in the present study showed almost equal percentage of monoterpene hydrocarbons and

oxygenated monoterpenes as mentioned in many other studies [20]. The predominance of 4-terpineol (oxygenated monoterpene) with percentage 48.7%, followed by γ-terpinene with percentage 10.4% was previously reported which is consistent with the current study [21].

In this study, we used combination of CO, LO, JO, and TTO to prepare herbal formulations and challenged their safety and efficacy in ameliorating the acne lesions. Many authors have reported the anti-inflammatory, antimicrobial, and antiacne activities of many essential oils [20]. The anti-inflammatory effect of TTO has been attributed to cyclic monoterpenes which reaches 80-90% of its content. The compound 4-terpineol, the main component in TTO, has been proven to reduce the production of IL-1, IL-8, IL-10, prostaglandin E2, and TNF. Also, it can modulate vasodilation and plasma extravasation. 4-Terpineol α- terpineol can suppress the production of superoxide by monocytes [22]. Three laboratory studies reported the antimicrobial activity of TTO by recording MIC 0.31-0.62% and 0.5% against one variant of *P. acnes* and MBC 0.25-0.5%. for 32 variants of *P. acnes*. Additionally, MICs of terpinen-4-ol and -terpineol were recorded 0.16–0.31% and 0.08–0.16%, respectively. against one *P. acnes* strain. Five of the studies evaluating the efficacy of topical application of 5% TTO for 4-8 weeks in reducing AV lesions ranged from 23.7% to 62.1% [23]. In the present study, we used only 1% of TTO which is consistent with the recommendation of the European Cosmetic Association to use no more than 1% TTO in cosmetic preparations [22].

The antimicrobial potential of LO was recorded to be positively correlated to its high and nearly equal content of linalool and linalyl acetate which are the main components in LO in our study [24]. (Mahmood et al., 2020). A study on a topical application of a mixture of 3% tea tree essential oil, 2% lavender essential oil and jojoba essential oil for 4 weeks showed significant improvement in the inflammatory and non-inflammatory acne lesions. Another study reported high efficacy of LO with a total inhibition of bacteria at concentration (250 mg/ml) [25].

The MIC and MBC of JO against *Cutibacterium acnes* were determined as 1 mg/ml and 2 mg/ml, respectively. The same study reported the significant improvement of JO antibacterial activity against *C. acnes* when combined with sub-inhibitory concentrations of chitosan, suggesting their use as adjuvants in the complex clinical situation of acne [26]. The incorporation of juniper oil in solid lipid microparticles for topical formulations decreased its evaporation and assisted the prolonged antibacterial and antiacne activity [27]. The combination of TTO and LO, which possess potential antibacterial and anti-inflammatory properties, with JO showed a synergistic impact that can effectively treat acne symptoms [28].

Many studies reported the the antioxidant activity and anti-inflammatory of CO that potentiate its application in acne management. CO exhibited significant antioxidant properties using free radical scavenging and β-carotene–linoleic acid assays and compared to ascorbic acid. Another study attributed its activity to the chamazulene content. Moreover, bisabolol activity to inhibit free radical formation in human skin fibroblasts has been reported. The anti-inflammatory activity of CO containing high percentage of α-bisabolol and farnesene, was like that of ibuprofen. Additionally, α-Bisabolol significantly inhibited TNFα and IL-6 which assisted in clinical decrease of skin edema in mice [29].

After confirming the safety of the formulation in vitro and testing its efficacy for the treatment of acne vulgaris on a sample of patients who reported no side effects , we recommend to assess the medications on a large scale of patients and comparing its efficacy with a well known traditionally used acne medications.

The authors don’t experience any conflicts of interest within this work.

All the contributors have revised and agreed on all information un the manuscript and each one had an exact role within this work.

Author contributions:
Heba got the idea and prepared the formulation, Sameh and Salsabeel analysed the data. **Shahenda** and Ragea applied the formulation to the patients and wrote the manuscript and Hagar arranged and revise the manuscript to be ready for submission.

5. References

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