Chemical Composition and Larvicidal Activities of Essential Oils of Medicinal Plants, Artemisia Sieberi and Tanacetum Balsamita Against Malaria Vector, Anopheles Stephensi

Samad Kazempour  
Tehran University of Medical Sciences  

Mansoreh Shayeghi  
Tehran University of Medical Sciences  

Mohammad Reza Abai  
Tehran University of Medical Sciences  

Hassan Vatandoost (✉ hvatandoost1@yahoo.com)  
Tehran University of Medical Sciences  

Masoumeh Pirmohammadi  
Tehran University of Medical Sciences  

Research

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Abstract

**Background:** The country is suffering from malaria disease. There are several chemical control of mosquito. Due to resistant of vectors to different pesticides, this research was conducted to measure the chemical components and larvicidal activities of two native plants, *Artemisia sieberi* and *Tanacetum balsamita* against *Anopheles stephensi*.

**Materials and methods:** Two species of medicinal plants were collected from different localities in Iran. The plant samples were dried in shaded place and Essential Oils (EO) dried over anhydrous sodium sulfate. The EOs was maintained in the dark sealed vials until conduction larvicidal tests. The maximum storing of EOs was two days. The larvicidal tests were carried out based on the guideline of WHO.

**Results:** The values of LC50 and LC90 of EOs were 47.9 and 178.8 ppm for *Artemisia sieberi*. The figure of 26.2 and 52.4 ppm was observed for *Tanacetum balsamita*. The chemical constituents of *Tanacetum balsamita* and *Artemisia sieberi* which showed the highest efficacy for larviciding. A total of 39 constituents were isolated from *Tanacetum balsamita*. The main constituents were Thujone (52.37%) and Carvone (26.84%). Totally 57 constituents were detected in *Artemisia sieberi* and the main components were: camphor (23.6%), 2-Ethyl-3-methyl maleic anhydride (15.193%) and, Bombykal (10.32%), Ethylbutenol (10.74%).

**Conclusion:** New formulations of plants should be prepared and then evaluated under semi-fielded and filed conditions in a malarious areas.

Background

Mosquitoes are serious public health problem and important pests in tropical and sub-tropical countries. *Anopheles stephensi*, a highly competent vector of plasmodium is considered a prominent vector of urban malaria [1]. Malaria is the main vector borne diseases worldwide. According to the recent record of World Health Organization, 228 million cases have been reported in 2018 mainly in African region [2]. Based on the report of Ministry of Health of Iran, less than 89 locally-transmitted cases in 2017 have been reported. The aim of country is to eliminate the disease by 2025 [3]. *An. stephensi* is the main malaria vector southern parts of country [4]. Vector control by conventional insecticides, personal protection and using repellents from mosquito bites are the best measures of reducing the transmission of these diseases to human. The chemical insecticides have various problems such as, development of resistance, environment pollution and toxic effects on human and non-target organisms [5]. Malaria control in the country is now based on use different insecticides. Distribution of impregnated bed net with permethrin. Using *Bacillus thurungiensis* and Temephos as larvicide. (CDC, Ministry of Health). According to the WHO (2016) [6] report, drug and insecticide resistance in the malaria parasites and Anopheles species is the main problem. In malarious areas of the world, current vector control measure is not able to control the diseases effectively. Therefore, there is a need to develop and replace these toxic insecticides with new and better ones which are more environmentally safe. In Iran there are some species of malaria
vectors including: *An. stephensi*, *An. dthali*, *An. culicifacies*, *An. fluviatilis*, *An. superpictus* s.l., *An. sacharovi*, *An. maculipennis* Complex (Fig. 1).

**Materials And Methods**

**Collection, identification and extraction of plants:** The plants were collected in East Azerbaijan Province, Northern Iran (Fig.2). Subsequently they were rapidly transported to the School of Public Health, Tehran University of Medical Sciences.

**Plant identification:** The plant was identified by experts in Department of Plant Sciences, Tehran University (Figs 3,4).

**Rearing of mosquito larvae**

Rearing and maintaining mosquito larvae was carried out in the Culicidae insectarium of the School of Public Health Tehran University of Medical Sciences under standard conditions. Early 3rd-4th instar of larvae were used for larvicidal activities.

**Biological tests (larvicidal)**

For larvicidal activities of EO of plants, the standard WHO method was used. Late 3rd - 4th instar larvae were used. In each test, 5 logarithmic concentrations of larvicide were chosen. A total of 4 replicates for each concentration and 2 replicated as control were considered.

**Statistical analysis**

The test results of evaluation of EO of plants were calculated after 24 hours exposure. The concentrations which cause 50 and 90% mortality were calculated as LC50 and LC90. Their confidence intervals and the regression line were measured using a regression probit analysis according to the Finney (1971) (7). The percentage mortality was calculated according to Abbot’s formula (1925) (8).

**Extraction of essential oil of plant**

Faculty of Pharmacology, Tehran University of Medical Sciences were involved in all plant extractions. Essential oils (EOs) of native medicinal plants were hydro distilled in a Clevenger- for 4-6 h and then anhydrous sodium sulfate were used for drying. The EOs were stored in the dark sealed vials at 4 °C until starting the experiment.

**Plant essential oils analysis**

Chemical composition of plant was analyzed using gas chromatography mass spectrometer. The plants components was performed by comparing their retention times and mass spectra from Wiley library [9].

**Results**
The chemical constituents of *Tanacetum balsamita* and *Artemisia sieberi* which showed the highest efficacy for larviciding. Results show a of 39 constituents from *T. balsamita*. The main constituents were Thujone (52.37%) and Carvone (26.84%) respectively. Totally 57 constituents were detected in the essential oil of *Artemisia sieberi* and the main components were: camphor (23.6%), 2-Ethyl-3-methyl maleic anhydride (15.193%) and, Bombykal (10.32%), Ethylbutenol (10.74%) (Table 1,2). The values of LC50 and LC90 of EOs were 47.9 and 178.8 ppm for *A. sieberi*. The figure of 26.2 and 52.4 ppm was observed for *T. balsamita* (table.3). Probit regression lines of two plants against larvae of *An. stephensi* is shown in Figs. 5,6.

**Discussion**

A total of 500 species of *Artemisia* are found worldwide. Asia has the greatest concentration of species, 35 species of the genus found in Iran. According to different investigations, it is used for the treatment of different diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses [10–13]. In the Middle east, the plant of *A. sieberi* is considered as famous medicinal plant for antihelmintic effects. The extractions of its flowers and leaves were used for treatment of gangrenous ulcers, infectious ulcers, and inflammations. *A. sieberi* is used as fodder for sheep for increasing of weight. It was used as carminative, to relieve inflammation and abscesses and to prevent leprosy. It is used as pharmacy in traditional medicine. It has antihelmintic, anti-spasmodic, antirheumatic, and antibacterial potency for the treatment of malaria, hepatitis, cancer, inflammation, and menstrual-related disorders [14]. The results of chemical composition analysis of *A. sieberi* essential oils showed the presence of β-thujone (0–11.6%), camphor (2.8–42.9%), 1,8-cineole (0.0-48.7%) and α-thujone (0-62.1%). (Mahboubi et al, 2015). *A. sieberi* extracts showed antimalarial effects against murine malaria [15].

The major constituents of the leaf oil of *T. balsamita* were bornyl acetate (47.7%), pinocarvone (27.1%), camphor (9.3%) and terpinolene (5.4%), while the flower oil contained bornyl acetate (55.2%), pinocarvone (34.2%), camphor (2.8%) and terpinolene (2.0%) and the stem oil contained bornyl actate (49.2%), pinocarvone (28%), camphor (9.5) and terpinolene (6%). [16]. Different local plants were identified and then their extractions were evaluated against *An. stephensi* in Iran [17].

**Conclusion**

Due to high larvicical activities of plants against malaria vector, *An. stephensi*, it is recommended to make appropriate formulations for these plants and evaluated them under field conditions.

**Declarations**

**Ethics approval and consent to participate:**

There is not applicable

**Consent for publication:**
Availability of data and material:

applicable

Competing interests:

The author declare that there is no conflict of interest

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**Tables**
| NO | tR  | compound                             | %   | RI  |
|----|-----|--------------------------------------|-----|-----|
|    |     | (Minutes)                            |     |     |
| 1  | 1.774 | Ethanol                             | 0.478 | -   |
| 2  | 2.111 | Hexane                               | 0.232 | -   |
| 3  | 6.503 | cis-Salvene                          | 0.398 | -   |
| 4  | 6.79  | 3,4-Octadiene, 7-methyl-             | 0.035 | -   |
| 5  | 10.28 | Comphene                             | 0.080 | 873 |
| 6  | 11.58 | beta-Pinene                          | 0.079 | 904 |
| 7  | 12.406| 1-Phenylethanol                      | 0.140 | 922 |
| 8  | 12.975| Yomogi alcohol                       | 1.599 | 933 |
| 9  | 14.002| p-Cymene                             | 0.257 | 955 |
| 10 | 14.15 | m-Cymene                             | 0.188 | 958 |
| 11 | 14.409| 1,8-Cineole                          | 1.352 | 963 |
| 12 | 15.711| gamma-Terpinene                      | 0.035 | 991 |
| 13 | 19.712| Thujone                              | 52.379 | 1067 |
| 14 | 20.046| 2,5-Octadiene                        | 1.298 | 1074 |
| 15 | 20.144| Artemiseole                          | 0.446 | 1076 |
| 16 | 20.461| trans-p-2,8-Menthadien-1-ol          | 0.242 | 1082 |
| 17 | 20.576| cis-3,7-Dimethyl-1,3,6-octatriene    | 0.260 | 1084 |
| 18 | 20.813| 6-Nonynoic acid                      | 0.349 | 1089 |
| 19 | 22.038| 2,6-Dimethyl-1,5-heptadien-4-ol acetate | 0.909 | 1113 |
| 20 | 22.648| 3-Cyclohexen-1-ol, 5-methylene-6-(1-methylethenyl)- | 0.208 | 1125 |
| 21 | 23.838| 2-Nonynoic acid                      | 1.796 | 1148 |
| 22 | 26.826| Carvone                              | 26.844 | 1211 |
| 23 | 27.456| Carvone oxide                        | 1.177 | 1229 |
| 24 | 27.932| Novatone                             | 1.264 | 1243 |
| 25 | 29.061| 4-Heptanone                          | 0.140 | 1277 |
| 26 | 36.012| Germacrene D                         | 1.015 | 1403 |
| NO | tR   | compound                                           | %   | RI  |
|----|------|----------------------------------------------------|-----|-----|
| 27 | 36.611 | tau-Muurolol                                       | 0.775 | 1420 |
| 28 | 37.201 | beta-Bisabolene                                    | 1.193 | 1438 |
| 29 | 37.8  | delta-Cadinene                                     | 0.369 | 1455 |
| 30 | 38.074 | alpha-Guaiene                                      | 0.196 | 1463 |
| 31 | 40.471 | Ledene oxide                                       | 0.717 | 1522 |
| 32 | 41.023 | Veridiflorol                                       | 0.151 | 1533 |
| 33 | 42.645 | alpha-Cadinol                                      | 0.783 | 1565 |
| 34 | 43.238 | 5-beta-H,7-beta,10-alpha-Selina-4(19),11-diene     | 0.783 | 1576 |
| 35 | 54.213 | n-Hexadecanoic acid                                | 0.167 | 1867 |
| 36 | 54.519 | 1,2-Longidione                                     | 0.599 | 1874 |
| 37 | 57.967 | 9-Octadecene                                       | 0.175 | 1955 |
| 38 | 58.953 | Isophytol                                          | 0.314 | 1977 |
| 39 | 60.79  | 4H-Naphtho[2,3-b]pyran-4,6,9-trione, 5,8-dimethoxy-2-methyl-(CAS) | 0.578 | 2020 |
### Table 2

**Chemical components of *Artemisia sieberi***

| NO | tR (Minutes) | compound                                                  | %    | RI   |
|----|--------------|-----------------------------------------------------------|------|------|
| 1  | 1.7          | Ethanol                                                   | 0.022|      |
| 2  | 1.765        | Acetone                                                   | 0.093|      |
| 3  | 1.975        | 2-Butenal                                                 | 0.067|      |
| 4  | 2.084        | Hexane                                                    | 0.510|      |
| 5  | 2.631        | Methacrylic acid                                          | 0.485|      |
| 6  | 3.178        | Ethylbutenol                                              | 0.035|      |
| 7  | 3.558        | 2,5-Octadiene                                             | 0.047|      |
| 8  | 4.247        | Butanoic acid, 3-methyl-, methyl ester                    | 0.043|      |
| 9  | 4.436        | 2-Methyl-3-butoenoic acid                                 | 0.070|      |
| 10 | 4.533        | 2-Hexenal                                                 | 0.034|      |
| 11 | 6.366        | Ethyl 2-methylbutanoate                                   | 0.061|      |
| 12 | 6.469        | Pentanoic acid, ethyl ester                               | 0.136|      |
| 13 | 8.833        | 5-Methyl-3-octyne                                         | 0.177| 837  |
| 14 | 9.053        | alpha-Pinene                                              | 0.202| 843  |
| 15 | 10.44        | Comphene                                                  | 4.142| 877  |
| 16 | 11.465       | Sabinene                                                  | 0.100| 902  |
| 17 | 11.566       | beta-Pinene                                               | 0.240| 904  |
| 18 | 12.397       | Ethanol                                                   | 0.419| 921  |
| 19 | 12.932       | Acetone                                                   | 0.118| 0.118|
| No. | RT  | Compound                                      | Mass   | MW  |
|-----|-----|----------------------------------------------|--------|-----|
| 25  | 17.189 | Butanoic acid, 3-methyl-, methyl ester       | 0.063  | 1018|
| 26  | 18.852 | Linalool                                     | 0.298  | 1051|
| 27  | 20.978 | Camphor                                      | 23.206 | 1092|
| 28  | 21.726 | Borneol                                      | 2.680  | 1107|
| 29  | 22.247 | 4-Terpineol                                  | 0.835  | 1117|
| 30  | 22.529 | Furfural                                     | 0.103  | 1122|
| 31  | 22.799 | alpha-Terpineol                              | 0.269  | 1128|
| 32  | 22.987 | Piperitol                                    | 0.641  | 1132|
| 33  | 23.727 | Terpinene-3-ol                               | 0.921  | 1146|
| 34  | 24.127 | 2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, cis- | 0.109  | 1154|
| 35  | 24.37  | Isobornyl acetate                            | 0.090  | 1159|
| 36  | 24.592 | Davana oil                                   | 0.337  | 1163|
| 37  | 25.289 | Carvol                                       | 0.630  | 1177|
| 38  | 25.991 | Piperitone oxide                             | 1.610  | 1191|
| 39  | 27.159 | Bornyl acetate                               | 0.124  | 1221|
|   |   | Description                                           |   |   |
|---|---|------------------------------------------------------|---|---|
| 40| 31.23| alpha-Copaene                                       | 0.427 | 1323|
| 41| 32.549| cis-Jasmone                                         | 1.422 | 1345|
| 42| 33.159| trans-Caryophyllene                                  | 0.204 | 1355|
| 43| 34.849| Perilla alcohol                                      | 0.497 | 1382|
| 44| 35.929| Germacrene D                                        | 0.665 | 1400|
| 45| 36.587| Germacrene B                                        | 0.928 | 1420|
| 46| 37.825| Davana ether                                        | 5.029 | 1456|
| 47| 38.757| 2-Ethyl-3-methyl maleic anhydride                    | 15.193 | 1484|
| 48| 39.879| Bombykal                                             | 3.281 | 1511|
| 49| 41.082| Bombykal                                             | 10.324 | 1534|
| 50| 41.496| Bombykal                                             | 5.984 | 1542|
| 51| 42.004| Humuladienone                                        | 0.309 | 1552|
| 52| 43.297| Isoaromadendrene epoxide                             | 0.674 | 1578|
| 53| 44.523| 4-[3-Methyl-1-(2-methyl-1-propenyl)-2-butenyl]-3-cyclohexen-1-one | 0.962 | 1603|
| 54| 44.971| cis-Davanone                                         | 1.179 | 1621|
| 55| 48.304| Ascaridole                                           | 1.274 | 1730|
| 56| 51.001| Benzyl salycilate                                    | 0.237 | 1793|
| 57| 54.213| n-Hexadecanoic acid                                 | 0.652 | 1867|
Table 3
LC50 and LC90 values of *Artemisia sieberi* and *Tanacetum balsamita* against larvae of *An.stephensi*.

| plant          | a       | b ± SE | LC50 (ppm) | LC90 (ppm) | χ² (heterogeneity) | χ² table (df) | p-Value |
|----------------|---------|--------|------------|------------|--------------------|---------------|---------|
| *Artemisia sieberi* | -3.7649 | 2.2405 | 41.3 | 141.2 | 40.828 * | 16.26 | 0.001 |
| *Tanacetum balsamita* | -6.0418 | 4.2586 | 23.1 | 46.2 | 3.863 * | 13.27 | 0.001 |