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Analysis of Extracts From Palestinian Inula Viscosa for Their Phenolic, Flavonoid and Lipid Contents, Antioxidant and Antibacterial Activity

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Abstract

Inula Viscosa is one of the most commonly used medicinal plants in countries of Mediterranean region. In this work extracts of different parts of Palestinian Inula viscosa (whole plant, leaves, stems and flowers) were obtained using two solvents (ethanol and methanol), and studied for their Total Phenolic Content (TPC), Total Flavonoid Content(TFC), lipid content 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging and antibacterial activities. Results had shown that Methanolic extracts of all plant parts had higher TPC, TFC and antioxidant activity than those of extracts obtained using ethanol. Among all extracts, the flower methanolic one had the highest TPC (173.15 mg EGA/g extract) and demonstrated the best antioxidant activity with EC50 of 12.47µg/ml. While the best total flavonoid content was observed for the methanolic extract from leaves (142.5 mg CE/g extract). The total lipid content for extract of all parts was also determined using hexane as extraction solvent, and Leaves showed the highest lipid percentage that is 14.37% from dry plant material. The antibacterial activity of the extracts was studied using agar dilution method on E-coli. For this solutions of extracts with concentrations (10 −2, 10−3 ,10−4 and 10−5 g extract/ml) in 8% aqueous DMSO were prepared and analyzed for their bacterial growth inhibiting activity. The tested extracts in the given concentration range exhibited 90-100% bacterial inhibition. In addition, a liquid hand soap enriched with Inula Viscosa extracts was prepared, and its antibacterial activity was tested both in vitro and in vivo. In vitro study the soap showed complete inhibition of E-coli, while in vivo tests resulted in 50-100% bacterial inhibition.

Key Words: Inula Viscosa, extraction, antioxidant activity, antibacterial activity

1. Introduction

Palestinian territories are covered with an extraordinarily diverse and huge number of plants. Palestinian Territories.

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About 47,000 living species have been identified in Palestine, with another 4,000 assumed to exist. Around 2,780 types of plants grow countrywide, from Alpine flowers on northern mountain slopes to bright red coral peonies and desert papyrus reeds in the south [1]. One important medicinal plant growing in Palestine is Inula Viscosa, which belongs to the Asteraceae family including about 100 species distributed in Asia, Europe, and Africa [2], predominantly, in the Mediterranean area. It has been used as herbal medicine since ancient Greek and Roman [3], and comprises several species of reputed medicinal value. For instance, Inula helenium L., Inula racemosa Hook.f. and Inula britannica L. are frequently used in ethnomedicine. Several Inula species are found in commercial herbal preparations, such as Inula helenium in the antiulcerous drug (Ventrofit), or supplements to medical applications as Pancreophyt, Relaxing Balm or Syrup of smokers [3]. This medicinal value of Inula can be due to the wide spectrum of active substances contained in it that are essential oils, fatty acids, phenols, flavonoids and a high content of sesquiterpenoids (about 400 sesquiterpenoids) [4] and some sesquiterpenic acids [2].

Inula viscosa is a strong smelling plant, woody at the base and having numerous yellow flowered heads at the top of the stem [3]. The leaves and stems of the plant are coated with a sticky resin secreted from glandular hairs growing on the surface of the leaves. In Morocco, Calabria (Southern Italy), Palestine, Algeria, Jordan and other countries decoction of Inula viscosa leaves and roots were used in traditional medicine for treatment of wide spectrum of diseases. However, the Palestinian Inula viscose species, are expected to be biologically more active than the European plants. In Palestine, the plants grow in warmer conditions with longer sunshine exposure and lack of rainfall. The longer period of exposure to pathogenic attacks during the hot summer is postulated to selectively enhance the local plants production of defensive phytochemicals [3, 5] that are responsible for the plant medicinal efficiency. The following Figure presents the Palestinian Inula Viscosa plant.

Palestinian Inula viscosa plant.

Extracts from Inula viscosa were found to possess a high content of bioflavonoids, Saponins, Sterols, Carotenoids, Sesquiterpene, Sesquiterpenoids and polyphenols, and thus they can serve as antioxidants, microbial and fungi inhibitors and anti-cancer agents [5-8]. Many studies were performed around the world on the methods and conditions of obtaining extracts from inula Viscosa [5, 7 , 9-10], in addition to investigating their wide spectrum of biological activities [8, 11-13]. However there are no researches concerning phytochemical analysis of extracts from the Palestinian plant. Therefore, the current study aims at extracting different parts of Inula Viscosa collected from Hebron mountains (Palestine) using different solvents with subsequent analysis of the extracts for their lipid, phenolic and flavonoid contents and antioxidant and antibacterial activities.

2. Material and Methods

2.1. Plant Material

Inula viscosa materials (whole plant, leaves, steams, flower, and roots) were collected from Hebron in November, 2015, washed and air dried in dark place for 15 days at ambient temperature. They were then ground into powder and stored -20 oC.

2.2. Chemicals

Ethanol, methanol, hexane, gallic acid, catechin, sodium carbonate, folin reagent, DPPH, tryptone glucose yeast extract agar, sodium hydroxide, aluminium chloride and sodium nitrite were all from Segma Aldritch company.

2.3. Extraction procedure

5-g samples of the dry powdered plant materials were extracted using 150 ml of two different solvents (ethanol or methanol). The extraction was performed by maceration and stirring for 48 hours at ambient temperature. Macerated extracts were filtrated and the solvents were evaporated using rotary evaporator at 50-55 oC till complete dryness.

2.4. Determination of Total Phenolic Content

Total phenolic content (TPC) was determined using Folin reagent according to the method described in literature [14]. 10.0 mg of the extract samples were dissolved in 10.0 ml of aqueous (80%) methanol to get the concentration of (1mg/ml). 0.5 ml of each sample was mixed with 2.5 ml of Folin reagent and 2.0 ml (7.5%) Na2CO3 and allowed to stand for 40 minutes.

It was then centrifuged for 10 minutes and the absorbance of solutions was measured using UV- spectrophotometer at λ = 760 nm. The absorbances of standard solutions of gallic acid with concentrations (0.05, 0.04, 0.03, 0.02 and 0.01) mg/ml were measured and the obtained calibration curve was used for the calculation of TPC in extracts as milligram of gallic acid equivalents per gram of dried material (mg GAE/g dried extract).

2.5. Determination of Total Favonoid Content

The total flavonoid content (TFC) was determined using AlCl3 according to the method described in literature [15]. 5.0 mg of each extract was dissolved in 10.0 ml methanol. 1.0 ml of each solution was mixed with 4.0 ml distilled water, 0.3 ml (5%) NaNO2 solution, 0.6 ml (10%) AlCl3 solution and 2.0 ml (1M) NaOH and allowed to stand for 6 minutes. The absorbance was measured at λ = 510 nm against water as blank. Standard solutions of catechin with concentrations (10, 20, 30, 50 and 100) mg/ml were used to construct calibration curve which was used for the calculation of TFC as milligram of catechin equivalents per gram of dry extract (mg CE/g dried extract).

2.6. Determination of Antioxidant Activity

The radical scavenging activity of both methanolic and ethanolic extracts against 2,2diphenyl-picrylhydrazyl (DPPH) radical was measured according to the method used by [16]. The crude extract was dissolved in methanol to get different concentrations (80.0, 60.0, 40.0, 20.0, 10.0 µg/ml). 4.0 ml of each solution was added to 1.0 ml of freshly prepared (DPPH) solution (0.2 mM) and allowed to stand for 30 min at ambient temperature. The absorbance was measured at λ = 517. The results were expressed as radical scavenging activity percentage of the DPPH according to the formula: **DPPH scavenging effect (%) = [(Ablank - Asample)/ Ablank] * 100**. Where Ablank and Asample are the absorbance of the control and extract solutions respectively. The extract concentration resulting in 50% radical inhibition (EC50) was then determined from the graph of free radical scavenging activity versus extract concentration and expressed as µg/ml.

2.7. Determination of total lipids

Exactly weighed 5 g of dried plant material were extracted using hexane. The extraction was done by maceration for 48 hours at ambient temperature. The solid plant residues were separated by filtration, the solvent was evaporated using rotary evaporator at 40 oC till constant weight. The obtained lipid material was weighed and the total lipids was calculated as a percentage from the dry plant material .

2.8. The Antibacterial activity

2.8.1. Microorganism

The microorganism that was used in the present study was a safety laboratory strain of Escherichia coli (DH5α) from the Applied Biology lab, where the microbial work was conducted.

2.8.2. Screening for antibacterial activity

Agar dilution method was used for screening the antibacterial activity of the extracts as follows:

2.8.2.1. Media preparation

24g of tryptone glucose yeast extract agar (TGY) was dissolved in 1L distilled water. After it was autoclaved for 15 minutes at 121oC and 15lb, it was transferred to 40 plates. Each plate containing 25 ml media. The essay was performed in triplicate.

2.8.2.2. Extract preparation

100.0 mg of each extract was dissolved in 10.0 ml of aqueous (8%) DMSO. Then, 10 fold serial dilution was performed to obtain solutions with the following concentrations: 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} g extract/ml. These solutions were stored at 4oC.

2.8.2.3. Antibacterial activity test of extracts

100 µl of each extract solution was spread on a plate and left to dry. Then 1 µl of bacteria was spread on each plate using 1 µl inoculation loop. The plates were Incubated aerobically at 37 oC for 48 hours. The number of colonies on each plate were counted manually. A plate containing aqueous (8%) DMSO was considered as a positive control to calculate the percent inhibition of bacteria. Colony Forming Unit (CFU), MIC and inhibition % were calculated.

2.8.3. Antibacterial activity of Liquid hand soap

2.8.3.1. Preparation of liquid hand soap

For preparing the soap to the aqueous phase (Distilled water and Glycerin) SLS and Cocamide MEA were added with intensive mixing until a clear solution was obtained, after what the color and perfume were added.

Then the needed viscosity of soap was achieved by adding sodium chloride and its pH was adjusted at 5.7 using citric acid. 100.0 mg of the extract was dissolved in 1.0 ml of aqueous (8%) DMSO. Then this solution was diluted by adding different volumes of the prepared soap to get the concentrations of $10-2$, $10-3$ and $10-4$ g extarct/ml.

2.8.3.2. The antibacterial test of the soap

In vitro study: The antibacterial activity of the soap was investigated using the same method used for the antibacterial activity test in section

2.8.2.3. **In vivo study**:

Touches from a randomly chosen volunteer hand were taken using 10µl loop and spread on a plate. The volunteer is then asked to wash his hand using one of the tested soap samples. Then another 3 touches were taken and spread on another plate. The plates were incubated upside down at 37 oC. The results were taken after 24 h and 48 h. A comparison between plates from the same volunteer before and after using the soap was done. The inhibition % is calculated by the equation:
Inhibition% = [(number of colonies on human hand before using the soap - number of colonies after using the soap) / number of colonies before] * 100%

3. Results and Discussions

3.1. Extraction

Many solvents are used for plant extraction, however we used the most commonly used two solvents: ethanol and methanol. Samples of different plant parts were macerated with stirring for 48 h, filtrated, and the solvent was evaporated till constant weight. The % yield of extract was found as (g extract/100g dried plant material) and shown in Table 3.1

Table 3.1: The % of extracts in plant part

Extract	Extract %
leaves ethanolic extract	12.3
stem ethanolic extract	4.6
flower ethanolic extract	9.6
whole plant ethanolic extract	3.1
leaves methanolic extract	16.02
stem methanolic extract	7.94
flower methanolic extract	11.12
whole plant methanolic extract	5.36

From the Table we can see that the yield varies from 3.1% to 16% with leaves methanolic extract having the highest extract percent (16.0%).

3.2. Determination of Total Phenolic Content (TPC)

The results for determining TPC in all parts of Inula viscosa are presented in Table 3.2. From the Table it is obvious that the levels of TPC varies depending on plant part and extraction solvent. The values of TPC in obtained extracts are found to be in the range 99-173, while the highest TPC was determined in flower methanolic extract. Methanolic extracts gave a higher TPC than those of ethanolic extracts for all parts of the plant. For ethanolic extracts leaves gave the highest TPC, followed by the whole plant extract then flower one, while stem had the lowest TPC among all ethanolic extracts. Methanolic extracts show different trend in TPC with flower having the highest TPC followed by whole plant then stems, while leaves had the lowest TPC. The value of TPC for ethanolic leaves extract (TPC = 149.8) in this study was significantly higher than those reported for the same species in literature [7] and [17], where the ethanolic extract of inula leaves collected from Tunis in May were found to have TPC of 103 [7]. This can be attributed by the difference in conditions (climate and soil) of plant growth and the season in which plant is collected.

Table 3.2: Total phenolic content of extracts

Plant extract	TPC (mg EGA/g dried extract)
leaves ethanolic extract	149.8
stem ethanolic extract	97.6
whole ethanolic extract	101.8
flower ethanolic extract	99.1
leaves methanolic extract	152.7
stem methanolic extract	154.4
whole methanolic extract	169.0
flower methanolic extract	173.1

3.3. Determination of Total Flavonoid Content (TFC)

The results of TFC determination are showed in Table 3.3. From Table we can see that TFC content varies depending on plant part and solvent. The values found to be in the range of 103.3-142.5. The highest value was obtained from methanolic extract of whole plant (142.5) . Methanolic extracts gave a higher TFC than those of ethanolic extracts for all parts of the plant which emphasizes the results of TPC. In addition, for both Methanolic and ethanolic extracts, the highest TFC was exhibited by leaves followed by whole plant, then flower extract and stem had the lowest TFC. It should be noted that the value of TFC for ethanolic leaves extract (TFC = 129.9) in the current work was also higher than those reported for the same species in literature [7] and [17] in which leaves ethanolic extact had TFC equal 84.92 and 29.34 respectively. This can be attributed to the same reasons mentioned in the previous section.

Table 3.3: The Total flavonoids content in extracts

Extract	mg CE / g dried extract
leaves ethanolic extract	129.9
stem ethnolic extract	97.4
whole ethanolic extract	127.2
flower ethanolic extract	105.1
leaves methanolic extract	142.5
stem methanolic extract	103.3
whole methanolic extract	135.7
flower methanolic extract	123.8

3.4. Determination of Antioxidant Activity of extracts

The antioxidant activity for all parts of Inula viscosa was determined using DPPH free radical scavenging method. The results were expressed as the minimum concentration of extract that gives 50% inhibition of DPPH radicals and presented in Table 3.4. From Table we can see that EC50 is in the range 12.4-41.4 (µg/ml).

Table 3.4: The antioxidant activity of Inula Viscosa extracts

Extract	EC50 (µg/ml)
stem ethanolic extract	38.2
leaves ethanolic extract	20.4
flower ethanolic extract	23.6
whole plant ethanolic extract	17.1
stem methanolic extract	17.4
leaves methanolic extract	18.5
flower methanolic extract	12.5
whole plant methanolic extract	14.6

According to the data in Table 3.4, methanolic extracts of all parts of plant exhibited better antioxidant activity than extracts obtained using ethanol as extraction solvent, what completely agrees with the results for TPC and TFC. Furthermore, the flower methanolic was the best antioxidant followed by whole plant, stem and leaves (the same trend was observed in the TPC results). However, for ethanolic extracts whole plant gave the best results followed by leaves, flower, stem. The DPPH radicals inhibition activity of leaves ethanolic extracts (EC50 = 20.4 µg/ml) was better than that in the work [7] in which EC50 was 23.33 µg/ml). These results and those concerning TPC and TFC of extracts from Inula Viscosa leaves, obtained in our work for Palestinian plant and comparing them with those in other studies, confirm the previously made assumption that Palestinian plant may have better antioxidant and antibacterial activities than the same species from other parts of the world.

3.5. Determination of Total Lipid Content

Total lipid content in all parts of Inula Viscosa was determined and the results are represented in Table 3.2. Leaves had the highest content of lipids followed by whole plant, stem and flower. The value of total lipid in leaves (14.38%) was higher than those for the same species obtained in literature [7] which had a value of 6.14% . The higher value of lipids may be due to the difference in solvents used in extraction. In literature [7] the extraction was done using chloroform : methanol solution (2:1), while in this study hexane was used as a solvent. Also differences in growing conditions of the plant and collecting season may affect the lipid content.

Table 3.5: The total lipid content

Plant part	Lipid %
leaves	14.38
stem	7.92
whole	8.12
flower	6.73

3.6. Determination of Antibacterial Activity of extracts

The currently available screening methods for the detection of antimicrobial activity of natural products fall into three groups that include bioautographic, diffusion, and dilution methods [18-20]. The bioautographic and diffusion methods are known as qualitative techniques since these methods will only give an idea of the presence or absence of substances with antimicrobial activity. On the other hand, dilution methods are considered quantitative assays once they determine the minimal inhibitory concentration [18]. Thus the agar dilution method was used in our study for determination of antibacterial activity of Inula Viscosa extracts against E-coli DH5α.
Table 3.6 shows the inhibition percent of E-coli using extracts solutions in 8% DMSO obtained from different parts of plant with different concentrations. All extracts and concentrations tested gave an inhibition % higher than 90%, while the concentration 10^{-2} g/ml for extracts for all parts resulted in 100% inhibition of bacteria.

In addition, whole plant and flower methanolic extracts with concentration of 10^{-3}g/ml showed an inhibition % of 98.36% and 99.91% respectively.

Table 3.6: Inhibition percent of E-coli by extracts of Inula Viscosa

Extract	Inhibition %			
	10^{-2} g/ml	10^{-3} g/ml	10^{-4} g/ml	10^{-5} g/ml
leaves ethanolic extract	100	97.64	97.18	97.2
stem ethanolic extract	100	97.99	94.8	94.4
Leaves methanolic extract	100	97.85	96.84	95.9
stem methanolic extract	100	92.5	91.69	91.5
flower methanolic extract	100	99.91	95.26	94.8
whole plant methanolic extract	100	98.36	97.64	95.4

3.6.2. The antibacterial activity of soap

The antibacterial activity of soap enriched with Inula Viscosa extracts was assessed both in vitro and in vivo against E-coli DH5α. The antibacterial activity was measured for soap samples containing different concentrations of extracts. **In vitro study**: The samples tested were whole plant methanolic extract and leaves ethanolic extract with concentrations 10−2 and 10−3 g/ml, in addition to leaves methanolic extract and stem methanolic extract with concentration 10−2 and 10−4 g/ml respectively. They all gave 100% inhibition. **In vivo study**: Different soap samples were tested in vivo using touches from human hands. The results were obtained after 24 and 48 of incubation. For determining the percent inhibition of bacteria for extract enriched soap samples, a media containing soap without any preservatives was used as a positive control. The results of this test are presented in Table 3.7. From table we can see that using stem methanolic extract at concentration 10−4 and leaves ethanolic extract at concentration 10−2 resulted in complete inhibition to bacteria.

Table 3.7: The Inhibition percent of Bacteria in vivo study

Extract (concentration)	Inhibition %
whole methanolic extract at concentration (10^{-3} g/ml)	85%
leaves ethanolic extract at concentration (10^{-3} g/ml)	83%
stem ethanolic extract at concentration (10^{-3} g/ml)	50%
flower methanolic extract at concentration (10^{-3} g/ml)	50%
stem methanolic extract at concentration (10^{-4} g/ml)	100%
leaves methanolic extract at concentration (10^{-2} g/ml)	93.75%
leaves ethanolic extract at concentration (10^{-2} g/ml)	100%

4. Conclusions

The results of the present work showed strong dependence of TPC, TFC, lipid content and the Antioxidant activity of the extracts of Inula Viscosa on the plant part. Both methanolic and ethanolic extracts obtained from all parts of Palestinian Inula Viscosa species collected in November from Palestine have higher levels of TPC, TFC and antioxidant activities than those obtained in other literature studies. Therefore, they can serve as potential source of valuable natural antioxidants. In addition, the extracts obtained exhibited a high antibacterial activity against E-coli DH5α. Also the enrichment of liquid hand soap with these extracts resulted in complete inhibition of bacterial growth. Thus they can be recommended as antimicrobial additives to such commercial products.

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6. References

Norman, A. B., Khalaf-Sakerfalke V. J. (2009). Flora and fauna in Palestine. Gazelle: The Palestinian Biological Bulletin, 91, 1–31.

Xiang-Rong, C., Chun-Hui, W., Pan-Lei, W., Xu-Feng, Z., Qi, Z., Shi-Kai, Y., Hui-Zi, J., Wei-Dong, Z. (2014). New sesquiterpenic acids from inula wissmanniana. Fitoterapia, 95, 139–146,.

Seca, A. M. L., Grigore, A., Pinto, D., Silva, A. M. S. (2014). The genus inula and their metabolites: From ethnopharmacological to medicinal uses. Journal of ethnopharmacology, 154(2), 286–310.

Xing-Yu, X., Peng, S., De-An, G., Xuan, L., Jun-Hua, L., Li-Hong, H. (2015). Cytotoxic sesquiterpene lactone dimers isolated from inula japonica. Fitoterapia, 101, 218–223.

Haoui, I. E., Derriche, R., Madani, L., Oukali, Z. (2015). Analysis of the chemical composition of essential oil from algerian inula viscosa (L.) aiton. Arabian Journal of Chemistry, 8(4), 587-590.

Haoui, I. E., Derriche, R., Madani, L. (2016). Extraction of Essential Oil from Inula viscosa (L.) Leaves: Composition, Antifungal Activity and Kinetic Data . Journal of Essential Oil Bearing Plants, 19(1), 108-118,

Mahmoudi, H., Hosni, K., Zaouali, W., Amri, I., Zargouni, H., Ben Hamida, N., Kaddour, R., Hamrouni, L., Ben Nasri, M., Ouerghi, Z. (2016). Comprehensive phytochemical analysis, antioxidant and antifungal activities of inula viscosa leaves. Journal of Food Safety, 36(1), 77-88.

Akkawi, M., Abbasi, I., Jaber, S., Aburemeleh, Q., Naseredin, A., Lutgen, P. (2014) Investigation of traditional Palestinian medicinal plant inula viscosa as potential anti-malarial agent. British Journal of Pharmacology and Toxicology, 5(5), 156– 162,.

Ali-Shtayeh, M. S., Yaghmour, R., Faidi, Y. R., Salem, K., AlNuri M. A. (1998). Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. Journal of Ethnopharmacology, 60(3), 265–271. Fontana , G., Rocca, S., Passannanti, S., Paternostro, M. (2007). Sesquiterpene compounds from *Inula viscose*. Natural Product Research, 21(9), 824-827.

Talib, W. H., Mahasneh, A. M. (2010). Antiproliferative activity of plant extracts used against cancer in traditional medicine. Scientia pharmaceutica, 78(1), 33- 45.

Wenqiao, W., Ben-Daniel, B. H., Yigal, C. (2004). Control of plant diseases by extracts of inula viscosa. Phytopathology, 94(10), 1042–1047.

Ali-Shtayeh, M. S., Yaghmour, R., Faidi, Y. R., Salem, K., AlNuri, M. A. (1998). Antimicrobial activity of 20 plants used in folkloric medicine in the palestinian area. Journal of Ethnopharmacology, 60(3), 265–271.

Feng Ma, J., Ryan, P. R., Delhaize, E. (2001). Aluminium tolerance in plants and the complexing role of organic acids. Trends in plant science, 6(6), 273–278.

Dewanto, V., Wu, X., Adom, K. K., Hai Liu, R. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of agricultural and food chemistry, 50(10), 3010– 3014.

Sun, J., Chu, Y., Wu, X., Hai Liu, R. (2002). Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry, 50(25), 7449–7454.

Trimech, I., Krisztina Weiss, E., Sanda Chedea, V., Marin, M., Detsi, A., Ioannou, E., Roussis, V., Kefalas, P. (2014). Evaluation of anti-oxidant and acetylcholinesterase activity and identification of polyphenolics of the invasive weed dittrichia viscosa. Phytochemical Analysis, 25(5), 421–428.

Valgas, C., Machado de Souza, S., Smˆania, E., Smaˆnia, A. (2007). Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology, 38(2), 369-380.

Zaidan, M. R., Noor Rain, A., Badrul, A. R., Adlin, A., Norazah, A., Zakiah, I. (2005). In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop Biomed, 22(2), 165–70.

Vlietinck, A. J., Berghe, D. V., Maes, L. (2006). Anti-infective potential of natural products. Journal of ethnopharmacology, 106, 290–302.