



ORIGINAL ARTICLE

Effects of Synergistic blend of three Plant Extracts of Yarrow, Wormwood and walnut Leaves the area Arsbaran- *In vitro* Leishmania Major in Tabriz, Iran

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ABSTRACT

Leishmaniasis is one of the major problems in health systems. Due to the side effects of medications used to treat these diseases and people also tend to use herbal medicines, This study aimed to investigate the effect of ethanol extracts of three plants, yarrow, wormwood and walnut leaf extracts on Leishmania major and mixed three synergistic effects in vitro In vitro study was done. According to the study, three plant extracts at concentrations of mg / ml 25 were prepared, then the solvent concentration in the culture media with parasites in PBS buffer plus two percent DMSO and injected at zero, 6, 24, 48 and 72 hours of infection were analyzed. In examining the effects of plant extracts to increase the idle parasites of parasites was determined that this inactivity is directly related to time, After 24 hours, so that meglumine antimoniate and amphotericin B after 30 minutes, compared with the effect of plant extracts, parasites were destroyed within 24 hours. The results showed that combining extracts of three plants listed on Leishmania is effective, Although further studies to demonstrate the effectiveness of volunteers in laboratory animals and patients seems necessary.

Keywords; *Leishmania, yarrow, wormwood, walnut leaves*

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INTRODUCTION

Leishmaniasis refers to a spectrum of disease caused by different species of Leishmania. Operating condition, Protozoon Leishmania name of the order Kinetoplastida, which according to both the environment and flagellated Leishman without flagella or visible object? This parasite in the vertebrate body, mononuclear phagocytotic cells live and multiply within them. Leishmania are transmitted by sand fly species (1).

It is estimated that about 12 million cases of cutaneous leishmaniasis is present in parts of the world And 350 million people are at risk of developing the disease. Currently 88 countries are infected with leishmaniasis. The incidence of cutaneous leishmaniasis in iran an average of about 35 per hundred thousand population (2). The widespread use of pharmaceutical products containing plant origin for various reasons such as fewer side-effects, Better patient acceptance and use of traditional medicine advised past generations And past generations and less expensive medicinal plants and also more compatible with the human body is a normal physiological function (3). The leaves of some plants such as walnut, Yarrow and wormwood are frequently used in traditional medicine have been numerous studies on their antimicrobial properties (4-7). So it was decided that in this study the effects of these plants on Leishmania and Trichomonas are reviewed and analyzed. Yarrow is a plant belonging to the Asteraceae family. In fact, this plant is the most popular herbs in abundance in ancient medicine to treat Diseases in general and, in particular, was used for wounds and burns (8). The most important compounds in this sector include volatile oil, Polyphenolic compounds, some types of flavonoids, lactones, betaine, styrene compounds, resins, tannins, Ashilin, Phosphate, nitrate, and potassium salts of organic acids. Studies on the plant represent the anticonvulsant effects (9), anti-inflammatory (10), antispasmodic (11), antibacterials, antifungals (12-14), analgesics (15), Blood pressure (16), lipid-lowering, anti-arrhythmias (17) and vegetable antioxidants (18). Walnut Juglans regia plant genus of the family Juglandaceae is. Walnut leaf extracts are bactericidal and germicidal effects. Walnuts and leaves skin tonic and blood purifier. The leaves and bark are particularly bitter because of the tannins in the treatment of cutaneous

and scrofulous diseases are advantageous. Walnut leaves for washing wounds and heal them very helpful and decoction of walnut leaves to treat headaches, frostbite and skin diseases. Fresh leaves of walnut vermin and insects such as moth destroy. Because copper is so nuts helps the body absorb iron, The decoction of walnut leaves or green bark is used to treat anemia and weakness (19). Herbal derivatives made from walnut in the topical treatment of acne, inflammatory diseases, ringworm and fungal infections, and bacterial and Viral applicable. Antibacterial, antifungal and antiviral chemical constituents of this plant by -5-hydroxy-1, 4 Nephtocoinon in it that causes a reaction with glutathione cycle and is regenerated (20). Citrus known as wormwood wormwood flowers the family is due to properties such as Insecticidal, appetizer, eliminate intestinal worms and also a bitter and aromatic compounds traditionally has been used in traditional medicine. The fragrant essential oil obtained from wormwood in some Food and beverages, in the pharmaceutical, agricultural, or as antibacterial, antipyretic, anti-fertility, anti-malaria and Cardiovascular amplifier is also due to the presence of toxic compounds in the plant that is used for the preparation of insecticides and plant Acaricide (21). Wormwood herbal component of ancient medicine is effective in treating malaria. Also this plant in the treatment and prophylaxis Schistosoma effect on China and Africa to treat Klunorkys is too cold. Inhibition of growth of Toxoplasma gondii and Leishmania treatment is also important uses of Wormwood, It also has anti-fungal (*Aspergillus fumigatus*) and its antimicrobial (anti-Leptospira) has been demonstrated (22). The therapeutic property of the plant is its flowers. The active compounds in extracts of this plant, aromatic compounds, And anti-inflammatory properties and anti-microbial monoterpene ketone (23), And Eucalyptus prepared, linalool, Caryophyllene, Pennines, camphor, butyl acetate, limonene, Sub-vinyl acetate, cis chrysanthenyl, Cis-epoxy Ocimene, Bvrnyl acetate and bitter and toxic compounds named Kamasolin and Tojon (24).

MATERIAL AND METHODS

Preparation of plant extracts:

Brushwood and unusable parts of plants taken apart and after cleaning, In special trays and dried at room temperature and crushed with an electric grinder and passed through sieve No. 10. The resulting powder mixture with a ratio of one to three with 80% ethyl alcohol to the extent that alcohol cm 3-2 on powder coat. During the soaking solution, although the Shaker hour 30 minutes to shake it out of its active ingredients. After 72 h, the solution was passed through filter paper Whatman No. 1. This procedure was repeated twice, each time for 24 hours. The resulting product to the exclusion of all alcohol by distillation and rotary evaporation under vacuum at 40 ° C condensing temperature and dry was placed in a 40 ° C oven. Yarrow, Wormwood leaves and walnuts were made ready for operation at a later stage.

Preparation and cultivation of Leishmania:

Leishmania major promastigotes code MRHO / IR / 75 / ER promastigotes of the Department of Parasitology, Faculty of Medicine, Tabriz University of Medical Sciences, which was held at -70 ° C were obtained. After melting the Stoke area (NNN) Novy Mac Neal Nicole moved to adequately grow. he bulk of the 1640-RPMI medium with antibiotics to kill the parasite was transmitted at a distance of 4-3 days were frequent. The mass production of stationary phase and transferred to fresh medium with 10% glycerol in appropriate vials Share for 24 h at 20 ° C and then were stored in 195 ° C.

Groups of the Leishmania

1. Glvkantym medium alone (MIC = 10 µg / ml (63)) and Leishmania two percent of dissolved PBS buffer plus DMSO (control -)
2. Medium containing amphotericin B alone (MIC = 0.7 µg / ml (64)) and Leishmania two percent of dissolved PBS buffer plus DMSO (control -)
3. Leishmania medium containing only two percent of dissolved PBS buffer plus DMSO (control +)
4. Leishmania-containing medium alone (control)
5. Leishmania culture and each of the three plant extracts at concentrations of mg / ml 25% of dissolved PBS buffer plus DMSO.
6. Leishmania culture medium and extracts of three plants each in two concentrations mg / ml 25 dissolved in PBS buffer solvent DMSO plus two percent Neubauer slides were used for counting of parasites. All areas at the beginning of culture (time zero) and at hours 6, 24, 48, 72 parasite viability and motility were assessed.

Methods of data analysis

According to a study variables were measured at successive times, from the GLM Repeated Measure ANOVA, a comparison within groups (within group), the Was used. The joint test with a significance level of 0.05 was considered significant. Data were analyzed by SPSS version 16.

RESULT

Studied the effect of plant extracts on Leishmania parasites are summarized in Table 1 have been reported. Repeated measures ANOVA showed a significant decreasing trend in the number of leishmania parasites in there time (0.05> P-value) which represents the effect of plant extracts on reducing the number of Leishmania parasites. This test also showed that the plant extracts have different effects (0.05> P-value). Multiple comparison tests showed that the plant effect of walnut leaves, yarrow plant is less than (0.05> P-value). Yarrow and wormwood plant, but both have the same effect (0.05 <P-value).

Table 1: Average number of Leishmania parasites in culture media containing compound studied at three plant extracts studied

The extract	Zero hour	6 hours	24 hours	48 hours	72 hours
Wormwood + Yarrow	206/7±81/4	33/3±14/4	13/3±12/6	0	0
Walnut leaves+ yarrow	206/7±81/4	120/3±26/4	90±26	66/7 ± 28/9	41/7±28/9
Walnut leaves + Wormwood	206/7±81/4	156/7±40/4	116/7±28/9	90 ± 26	66/7±28/9
The three plant extracts	206/7±81/4	190±52/9	140±52/9	116/7±28/9	90 ± 26

Values are reported as mean ± SD. All results × 10⁴

Table 2 Average number of Leishmania studied at concentrations in culture media containing a mixture The three plant extracts tested in this study shows. As mentioned earlier, according to figures obtained Ic50 concentrations used in this section, including the concentration of 25 mg/ml For each plant, yarrow, wormwood and walnut leaves after the injection concentration and the number of parasites was determined at different times That the combined effect of wormwood and yarrow extracts on Leishmania compared with controls was statistically significant and there was no significant effect of other compounds.

Table 2: Average number of Leishmania parasites in culture media containing compound studied at three plant extracts studied

The extract	Zero hour	6 hours	24 hours	48 hours	72 hours
Wormwood + Yarrow	206/7±81/4	33/3±14/4	13/3±12/6	0	0
Walnut leaves+ yarrow	206/7±81/4	120/3±26/4	90±26	66/7 ± 28/9	41/7±28/9
Walnut leaves + Wormwood	206/7±81/4	156/7±40/4	116/7±28/9	90 ± 26	66/7±28/9
The three plant extracts	206/7±81/4	190±52/9	140±52/9	116/7±28/9	90 ± 26

Values are reported as mean ± SD. All results × 10⁴

Diagram is presented in Figure 1. As can be seen Ic50 Yarrow concentration 25 mg / ml The plant extract after 24 hours almost destroyed half of Leishmania parasites is based on a review of synergistic effect of the plant extract In this study, the concentration of Leishmania 25 mg / ml is used for the plant.

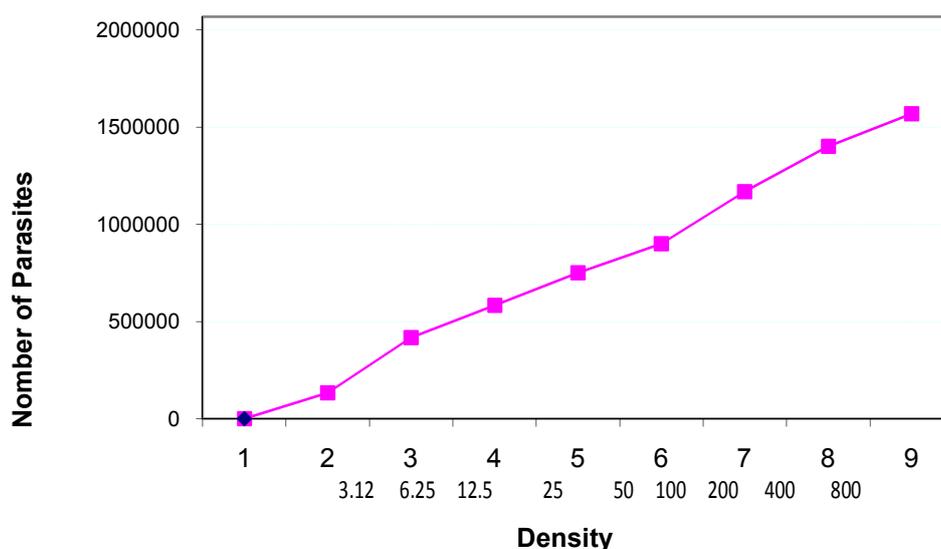


Figure 1: Diagram Ic50 effect of different concentrations of the plant extract on Leishmania

Ic50 diagram in Figure 2 and is based on the concentration of the extract of wormwood 25 mg / ml Wormwood herb extract in 24 hours, it destroyed half of Leishmania parasites. Therefore, the synergistic

effect of the combination of all three plant extracts at the concentrations used in this study for the herb wormwood.

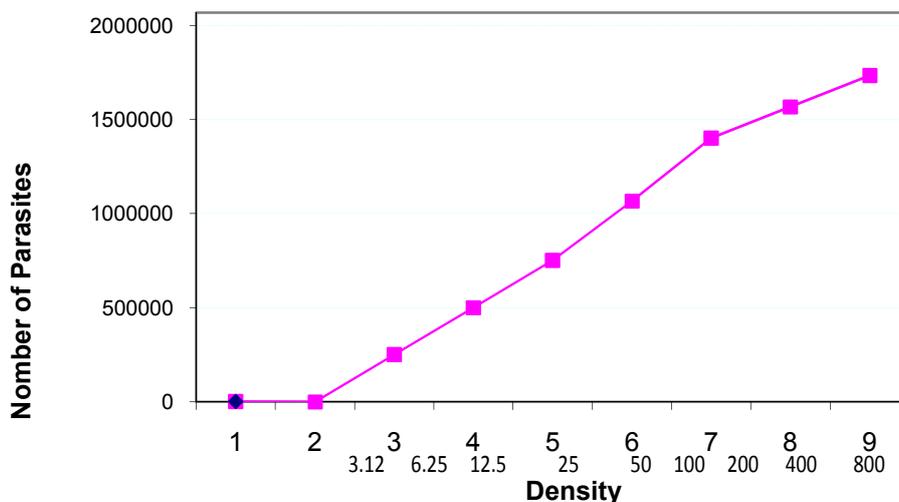


Figure 2: Diagram Ic50 effect of different concentrations of wormwood extract on Leishmania

Figure 3 shows a diagram Ic50 walnut leaves and is seen as one half of Leishmania parasites at concentrations of 25 mg / ml The plant extract was gone after 24 hours on the basis of the review of synergistic effect of the plant extract In this study, the concentration of Leishmania 25 mg / ml for walnut leaf has been used.

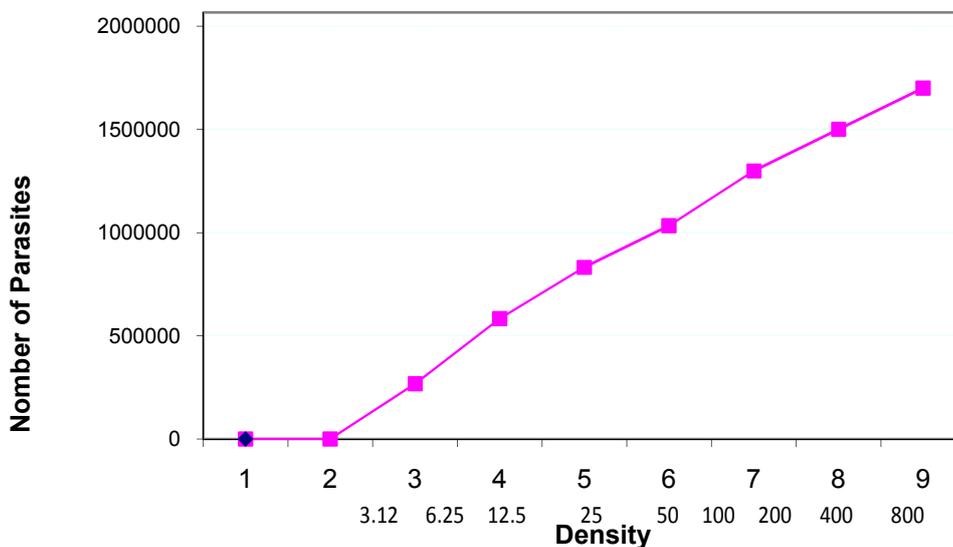


Figure 3: Diagram IC50 effect of different concentrations of walnut leaf extract on Leishmania

DISCUSSION AND CONCLUSIONS

Observed increase in the number of Leishmania parasites in the study area (no Yarrow) was significant. While investigating the effect of different concentrations of the plant extract on Leishmania revealed that all concentrations this extract has been studied to reduce the number of Leishmania parasites Concentrations in comparison with the control, given the results of the effect of decreasing concentrations of 100, 200, 400 and 800 are significant. (P-value <0.05) and the other concentrations (3/1,6/25,12/5,25 and 50) reduced the number of parasites Medium was not significant. (P-value> 0.05) to evaluate the effect of walnut leaf extracts on parasite Leishmania in the control (without extract of *Juglans regia*) increase in the number of parasites was observed that This increase was statistically significant at concentrations (25, 50, 100, 200, 400 and 800) extracts Reduce the number of Leishmania parasites is

statistically and compared these concentrations with the According to the results, the only effect of decreasing concentrations of 200, 400 and 800 in the comparison was significant. (P-value <0.05) and the size concentrations (3/1, 6/25, 12/5, 25, 50 and 100) reduced the number of parasites compared with controls was not significant. (P-value > 0.05).

Effect of different concentrations of wormwood extract was determined by the concentration of the extract on Leishmania parasites has reduced the number of All these effects are statistically and compared with the effect of decreasing concentrations of 50, 100, 200, 400 and 800 was significant. (P-value <0.05) in size and concentration (3/1, 6/25, 12/5 and 25) reduced the number of parasites was not significantly different with respect to the environment. (P-value > 0.05). The study compared the effect of plant extracts on Leishmania parasites trend over time was observed a significant decrease in the number of (0.05 > P-value) that represents the effect of plant extracts on reducing the number of Leishmania parasites. Plant extracts have also been shown to have different effects (0.05 > P-value) and determined that the herb yarrow herb walnut leaf has less influence (0.05 > P-value), but the herb yarrow and wormwood have the same effect. (0.05 < P-value) Savoia and colleagues impact various materials such as propolis on the growth of Leishmania major in the In-vitro study of the effects of various concentrations observed in Leishmania (25). Mustonen and colleagues in a study of the role of propolis on Leishmania donovani Amastigote killing by nitric acid and TNF release from macrophages have pointed out in In vitro (26). Klinghardt and colleagues on the role of propolis in controlling diseases such as trichomonas Protozoery, amebiasis, and Giardiasis pointed Toxoplasmosis (27). Shirani and colleagues investigated the effect of hydrocarbon extraction Alcohol thyme, yarrow and propolis on healing the wounds caused by the parasite Leishmania, the effectiveness of these extracts on wound healing have been reported (7).

Carpenter and Associates study investigating the effects of essential oil of Artemisia (Artemisia sieberi) on wounds Leishmania major in mice showed complete recovery after 30-day treatment period in any Which of the treated mice was observed at concentrations of sagebrush and microscopic examination of samples taken from the mice studied were positive. Artemisia at levels 1, 3 and 5% is not effective in the treatment of leishmanial ulcers and reducing the diameter of the emulsion Artemisia wounds and cuts Significant leishman bodies after the end of treatment (28). Statistics and Associates study results showed that the purified extract of Vinca plant at different concentrations A significant decrease in the number of Leishmania major promastigotes. Use of this purified extract of the chloroform phase, an injection to prevent the development of ulcers caused by Leishmania major in Balb / c mice compared to untreated control was very effective, so that statistically significant differences (0.05 > P-value) is (29). Talari et al.'s Study the effect of different concentrations of the agent of visceral leishmaniasis in Hedera Helix extract and glucantim In vitro revealed All tubules parasites were alive in the days of live parasites and the percentage viability at different concentrations differed glucantim, So that the concentration of 50 mg per ml after one day, only 2.5% They destroyed and at concentrations of 25 mg extract all the parasites die out after a day of love. In this study, different concentrations of the same concentrations glucantim to have more effect on parasite killing, and this difference was statistically significant (0.05 > P-value) (30). There was a significant effect of these plants on Leishmania. Study the effects of three plants such as yarrow, wormwood and walnut leaves was observed in Leishmania. (0.05 > P-value).

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