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Anti-leishmanial Activity of *Hyssopus officinalis*: A Review

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Abstract

Through this work shed light on the importance of medicinal plants and natural products, which are important sources of biologically active substances of medical interest? And especially shed light on *Hyssopous officinalis*. Nevertheless, several experimental studies are required to confirm the therapeutic potential of this plant and determine whether biological differences reflect the different isolation procedures, different types of plant material used, phytochemical constituents or different chemotypes.

Keywords

Hyssopous officinalis, Medicinal Plants, Anti-leishmanial Activity

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1. A Brife History and Uses of *Hyssopous officinalis*

Hyssopus officinalis L. is an important medicinal plant native to central and Southern Europe, Western Asia, and North Africa. The herb is an evergreen perennial plant with small, linear leaves and purplish-blue flowers [1, 2]. It is commonly used in folk medicine. Hyssop oil may be found as flavor ingredient in many food products. The oil is a fragrance component in soaps, cosmetics and perfumes [3]. Hyssop, rich in volatile oil, flavonoids, tannins, and marrubin, has been used as a healing herb to alleviate digestive disorders, cure laryngitis, or accelerate wound healing in Turkish folk medicine. It relaxes peripheral blood vessels and promotes sweating. It is also used as an expectorant, carminative, anti-inflammatory, anti-catarrhal, and antispasmodic in traditional medicine in many parts of the world [4]. It is further reported that certain fractions of hyssop (one being a polysaccharide designated as MAR-10) may inhibit the activity of the human immunodeficiency virus (HIV) [5, 6]. As a medicinal herb, hyssop is used in viral infections such as colds, coughs, sore throats, bronchitis and asthma. A tea made from the herb is effective in nervous disorders and toothache [3]. The oil is antimicrobial, mildly spasmolytic and exhibit strong antiviral activity against HIV [5]. Antibacterial, anti-fungal and antioxidant property of hyssop has been attributed to the presence of pinocamphone, iso-pinocamphone and β-pinene. Antiviral activity has probably been attributed to the presence of caffeic acid and tannins [5-8]. Moreover, fresh herb of hyssop is also characterized by a high content of vitamin C [9]. Hyssop has soothing, expectorant, and cough suppressant properties. The plant also includes the chemicals thujone and phenol, which give it antiseptic properties. Antimicrobial, antifungal, anti-protozoal and anticancer effects of Hyssop extract have been also reported [10]. Several essential oils have been used as therapeutic agents since ancient times, and some of them have been scientifically proven to possess medicinal properties, including anti-inflammatory [11], antiviral [12], antitumor [13], cytotoxic [14] and antimicrobial activities [15].

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2. Essential Oil of *Hyssopous officinalis*

Essential oils are complex mixtures of volatile, lipophilic and odiferous substances from the secondary metabolism of plants. They are mainly composed of monoterpenes, sesquiterpenes and their oxygenated derivatives (alcohols, aldehydes, esters, ketones, phenols and oxides). The bicyclic monoterpene ketones pinocamphone (trans-pinocamphone) and iso-pinocamphone (cis-pinocamphone) are generally known as the main characteristic components of the oils of *Hyssopus officinalis* [16] contributing approximately 36 to 41% of the total extract, which have to be taken into account due to their toxicity problems. Furthermore, the level of cis-pinocamphone recommends 34.5-50%, 5.5-17.5% of of the second isomer of pinocamphone (trans-pinocamphone) and 13.5-23% of β-pinene [16]. Pinenes and bicyclic terpenes can be found in the essential oils of coniferous trees (pine), rosemary, lavender, and turpentine [17]. These compounds may exhibit differences in toxicity and biological activity [18, 19]. Pinenes have two active constitutional isomers: α- and β-pinene. The racemic mixture is present in some essential oils, such as eucalyptus oil [17-19]. Pinenes show fungicidal activity and have been used for centuries to produce flavors and fragrances. Several biological activities are associated with pinenes, including use as a natural insecticide and antimicrobial activity [17]. Garg et al. [20] found that the main volatile constituents of *H. officinalis* essential oil from a variety of locations included β-pinene, limonene, β-phellandrene, 1,8-cineole, pinocamphone, iso-pinocamphone, pinocarvone, germacrene-D and methyleugenol. They also found that the oils extracted from different subspecies or plant populations of varying origin or morphology differed in percentage composition of the major volatile constituents. Similarly, Kizil et al. [4] found that the main compounds of the hyssop oil were isopinocamphone, β-pinene, 4-carvomenthenol, γ-terpinene, carvacrol, and pinocarvone, and these six components together constituted 75-81% of total essential oil. It is wellknown that most spices possess a wide range of biological and pharmacological activities. These volatile compounds are widely used in cosmetics as fragrance components, in the food industry to improve the aroma and the organoleptic properties of different types of foods, and in a variety of household products. In addition to their particular aroma, many essential oils and their isolated components also exhibit muscle relaxant, antibacterial and antifungal activities [21]. In some studies, the major components were isopinocamphone (57.27%), β-pinene (7.23%), terpinene-4-ol (7.13%), pinocarvone (6.49%), carvacrole (3.02%), p-cymene (2.81%) and pinocamphone (2.59%). These seven components constitute 86.54% of total oil [12]. In the literature, iso-pinocamphone, pinocamphone, β-pinene and pinocarvone were reported to be the most abundant components in hyssop oil [4, 21]. The composition of the essential oil of *H. officinalis* has been examined previously by Svoboda et al [4]. Other studies, reported that hyssop essential oil could be categorized depending upon their percentage composition of β-pinene, limonene, pinocamphone, and iso-pinocamphone [8, 16, 20, 22- 26]. These results are in agreement with some of the previously published data. Different compounds have been identified as the main component in hyssop oil by other researchers: methyleugenol (38%) by Gorunovic et al. [27], 1,8-cineole (53%) by Vallejo et al. [24], pinocamphone (49.1%) by Garg et al. [20], isopinocamphone by Mitic and Dordevic [26], and pinocarvone (36.3%) by Ozer et al. [8]. Also, Said-Al Ahl et al. [28] found that , the major constituents of *H. officinalis* oil were cis-pinocamphone (26.85 %), β-pinene (20.43 %), trans-pinocamphone (15.97%), α-elemol (7.96 %), durenol (3.11%), β-phellandrene (2.41%), caryophyllene (2.34%), (E)-2,6-dimethyl-1,3,5,7-octatetraene (2.27%), 3(10)-caren-4-ol,acetoacetic acid ester (2.14%), bicyclogermacrene (1.83%), myrtenol (1.73%), germacrene-D (1.68%), limonene (1.56%), γ-eudesmol (1.36%) and linalool (1.08%).

3. Phytochemical Constituents

From the literatures, there are several chemical compounds that isolation of *H. officinalis.* The phytochemical study of the aerial parts of *H. officinalis* cultivated in Xinjiang, China, revealed isolation of two new flavonoid glycosides and nine other known flavonoids from the ethanolic extract of the plant. The new compounds were identified as; quercetin 7-O-b-Dapiofuranosyl-(1→2)-b-dxylopyranoside (1) and quercetin7-O-b-D-apiofuranosyl-(1→2)-b-D-xylopyranoside 30-Ob-D-glucopyranoside (2), together with nine known flavonoids apigenin (3), apigenin 7-O-b-Dglucopyranoside (4), apigenin 7-O-b-Dglucuronopyranosidemethyl ester (5), luteolin (6), apigenin 7-O-b-D-glucuronide (7), apigenin 7-O-b-Dglucuronopyranosidebutyl ester (8), luteolin 7-O-b-Dglucopyranosid (9), diosmin (10) and acacetin 7-O-a-Lrhamnopyranosyl-(1→6)-b-D-glucopyranoside (11). The free radical scavenging activity of the compounds 1 to 11was also determined using 2,2-diphenyl-1-picrylhydrazyl(DPPH). The isolated compounds were found to possessnoble radical scavenging activity. Out of the isolated compounds, 1, 2, 6 and 9 with IC50 values in the rangeof 2.81 to 10.41 mmol/L exhibited stronger scavenging activity on DPPH assay than butylatedhydroxytolueneand L-ascorbic acid as standards [29]. Mario et al. [30] revealed the presence of the mostwidespread class of secondary metabolites, flavonoids, in *H. officinalis*. The major flavone, diosmin, was present in sepals, leaves, stems, rootsand whole plant. The other

identified compound in the plant was considered to be isoferulyl D-glucoseester [31]. Previously, Hilal et al. [32] reported isolation of seven glycosides of flavanone type from *H. officinalis* where the aglycon of the glycosideswere determined as 5,4'-dihydroxy-7,3'-dimethoxyflavanone. Considering the findings of the study, methanolic extract of the *H. officinalis* was shown to be rich in phenolic compounds, especially high in chlorogenic, protocatechuic, ferulic, syringic, p-hydroxybenzoic and caffeic acids followed byvanillic, p-coumaric, rosmarinic and gentisic acids [33-36]. Elsewhere, the presence of caffeic acid and its derivatives in the roots of *H. officinalis* cultivated in Romania was reported. Additionally, rosmarinic acid, ferulicacid and phenylpropanic compounds were also identified [37]. Later, Proestos et al. [38] identified the phenolic compounds for *H. officinalis* plant extracts. The most abundantphenolic acids in *H. officinalis* were considered to beferulic acid (13.2 mg/100 g of dry sample) and caffeic acid (6.5 mg/100 g of dry sample). Moreover, syringic, gentisic and p-hydroxybenzoic acids along with two flavonoids (+) catechin and apigenin were also detectedin *H. officinalis* [38].

Triterpenoids are one ofthe most abundant class of compounds in *H. officinalis*. It has frequently been suggested that triterpenoids play a defensive role against pathogens and herbivores. They also have several interesting pharmacological activities that include anti-inflammatory [39], anti-mycobacterial [40], antiviral [41] and cytotoxic [42] properties. For example, ursolic acid showed cytotoxic activity against lymphatic leukemia cells P-388 and L-1210 as well as human lung carcinoma cells A-549 [43]. Ursolic acid is also referred toas being a strong inhibitor of tumor promotion inmouse skin [44]. Liu [45] showed that both ursolic acid and oleanolic acid have anti-hyperlipidemic properties and wereshown to be effective in protecting against chemicallyinduced liver injury in laboratory animals. Skrzypek and Wysokinska [46] identified two sterols i. e. β-sitosterol (1) and stigmasterol (2), as well as several known pentacyclic triterpenes with an oleanene and ursene skeleton. The triterpenes were identifiedasoleanolic acid (3), ursolic acid (4), 2α, 3_-dihydroxyolean-12-en-28-oic acid (5), 2α, 3_-dihydroxyurs-12-en-28-oic acid (6), 2α, 3_, 24-trihydroxyolean-12-en-28-oic acid (7), and 2α,3,24-trihydroxyurs-12-en-28-oic acid (8). Compounds 5D8 were isolated as their acetates (6, 8) or bromolactone acetates (5, 7).

4. Anti-leishmanial Activity of *Hyssopus officinalis*

As a vector-borne parasitic disease, leishmaniasis has a spectrum of clinical manifestations from self-healing skin ulcers, mucosal damages to serious visceral infections [47]. According to the world health organization, leishmaniasis is one of the most neglected re-emerging and uncontrolled tropical diseases. The HIV and leishmaniaco infection is quickly growing in number in countries where leishmania species are endemic. The disease is reported from many parts of the world with an estimated prevalence of about 12 million cases. However, the incidence of *cutaneous leishmaniasis* (CL) and visceral leishmaniasis is estimated to be about 1.5–2 million and 500 000 new cases each year, respectively [48] and [49]. Upon inoculation in the dermis, the leishmanial promastigotes are phagocytosed by macrophages before interacting with extracellular matrix components to produce variable clinical syndromes [50]. In developing countries, the medicinal plants have long been used for disease treatment because they are safe and available at low price. Given the various beneficial drugs derived from medicinal plants, discovering new sources of drugs against leishmania infection would be of high significance [51-53].

Leishmaniasisis transferred by sand flies belonging to the genus *Phlebotomus*. It is found in most tropical and subtropical countries, but 90% of the estimated 1.5 million new cases each year happen in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria, where it is often associated with destitution [54, 55].

Akhlaghi et al [56] discussed that leishmaniasis is a group of tropical diseases caused by anumber of species of protozoan parasites. It is also regarded that presently there exists a population of 350 million ofpeople under risk of infection. Many therapeutic modalities have been used for the treatment of *cutaneous leishmaniasis*. Pentavalent antimonials such as sodium stibogluconate, have been the base for therapy in the endemic regions because of its efficacy and cost effectiveness. The disadvantages of the anti-monials are their requirement for intramuscular orintravenous injection each day for 20-28 days, their toxicity and the growing incidence of resistance in endemic and non endemicareas. Due to the limited availability of effective pharmaceutical products, most people in areas where leishmaniasis is endemic depend largely on popular treatments and traditional medicines to alleviate the symptoms. Inaddition to the various methods already mentioned, the treatment of leishmaniasis following the traditional medical practices of different cultures depends heavily on the use of native plants [57, 58]. To develop a suitable semi solid antileishmanial preparation, an ointment base of the extractwas prepared. White soft paraffin [petrolatum] was selected as a typical oleaginous ointment base in view of its wide spread use for many pharmaceutical ointments and lanolin was added to increase the hydrophilicity of the vehicle. DMSO is a well known penetration enhancer and was used to increase percutaneous

absorption of the drug. Our study showed high efficacy of herbals against leishmaniasis in vivo. The results were suggestive that *Hyssopous officinalis* plants have antiparasitic activity against L. major. Recent investigations focused on plants have shown an alternative way to get a potentially rich source of drug candidates against leishmaniasis, in which effective alkaloids, quinones, iridoids, terpenes, indole analogues have been found. Many compounds, including alkaloid, quinones, iridoids, terpenes, indole analogues have been documented to have anti-leishmania activity *in vitro*. Akhlaghi et al [56] Maleki et al [59] suggested that *Hyssopus officinalis*is effective for treatment of *cutaneous leishmaniasis* in mice.

Rosa et al [60] showed that Parasites of the genus Leishmania are transmitted by the bite of sand flies and infect cells of the mononuclear phagocyte lineage of their vertebrate hosts [61, 62]. Depending both on the virulence factors of the parasite itself and on the immune response established by the host, a spectrum of diseases known as leishmaniasis can appear, and these can be cutaneous and/or visceral [63]. Approximately 350 million people live in areas of active transmission of leishmania, with 12 million people throughout Africa, Asia, Europe, and the Americas directly affected by leishmaniasis. More than 90% of the cutaneous cases appear in Afghanistan, Saudi Arabia, Algeria, Brazil, Iran, Iraq, Syria, and Sudan [64]. Cutaneous leishmaniasis either can resolve spontaneously after a few months or, depending on the causative Leishmania species, can evolve into diffuse cutaneous, relapsing cutaneous, or mucocutaneous leishmaniasis, while untreated visceral leishmaniasis leads to death in the majority of patients [61]. *Leishmania amazonensis* is one of the principal agents of diffuse cutaneous leishmaniasis, which is usually unresponsive to all treatments known to date [65]. Also, visceralization of *Leishmania* strains that are classically restricted to *cutaneous leishmaniasis* has often been observed in patients with leishmania-human immunodeficiency virus co-infection [66].

The control of leishmaniasis remains a problem because no vaccines exist and the available chemotherapy still relies on the potentially toxic pentavalent antimonials, which cause serious side effects and require long-term treatment [67]. The rise in the rates of in vitro antimonial resistance due to intermittent drug exposure [68, 69], the isolation of antimonial-resistant leishmania strains from patients with unresponsive cutaneous leishmaniasis [61, 70], and recently, the numerous cases of visceral leishmaniasis among patients infected with the human immunodeficiency virus [66] make the search for new agents for the treatment of leishmaniasis urgent. Extensive studies of new drugs with antileishmanial activities, including both natural products and synthetic compounds, have been undertaken worldwide [61], although problems with the side effects of the chemotherapies used at present have not yet been solved. In recent years, there has been growing interest in alternative therapies and the use of natural products, especially those derived from plants.

References

[1] Lawless J (2002). The Encyclopedia of Essential Oils. Thorsons, 110-11.

[2] Wesolowska A, Jadczak D, Grzeszczuk M (2010). Essential oil composition of hyssop (*Hyssopus officinalis* L.) cultivated in north-western Poland. Herba Polonica, 56 (1): 57-65.

[3] Kizil S, Toncer O, Ipek A, Arslan N, Saglam S, Khawar KM (2008). Blooming stages of Turkish hyssop (*Hyssopus officinalis* L.) affect essential oil composition. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 58(3): 273-279.

[4] Kreis W, Kaplan MH, Freeman J, Sun DK, Sarin PS (1990). Inhibition of HIV replication by *Hyssopus officinalis* extracts. Antiviral Research, 14: 323-337.

[5] Gollapudi S, Sharma HA, Aggarwal S, Byers LD, Ensley HE, Gupta S (1995). Isolation of a previously unidentified polysaccharide (MAR-10) from *Hyssopus officinalis* that exhibits strong activity against human immunodeficiency virus type 1. Biochemical and Biophysical Research Communications, 210: 145-151.

[6] Letessier MP, Svoboda KP, Walters DR (2001). Antifungal activity of the essential oil of hyssop (*Hyssopus officinalis*). J. Phytopathology, (149): 673-8.

[7] Ozer H, Sokmen M, Gulluce M, Adiguzel A, Kilic H, Sahin F, Sokmen, A, Baris O (2006). In vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of *Hyssopus officinalis* L. SSP. Angustifolius. Ital. J. Food Sci., (18): 73-83.

[8] Grzeszczuk M, Jadczak D (2009). The estimation of biological value of some species of spice herbs. Acta Horticult., (830): 681-6.

[9] Kermanjani A, Jalalizadegan B, Tabatabaie F (2015). Comparison of *Hyssopus officinalis*, *Tussilage farfara*, *Carum copticum*extracts versus systemic glucantime in the treatment of *Cutaneous leishmaniasis* in Balb/c Mice. Advanced Studies in Biology, 7 (2): 49-54.

[10] Koudou J, Abena AA, Ngaissona P, Bessiere JM (2005). Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*. Fitoterapia, 76: 700-703.

[11] Loizzoa MR, Saabb A, Tundisa R, Stattia GA, Lamprontic I, Menichinia F, Gambarid R, Cinatle J, Doer HW (2008). Phytochemical analysis and in vitro evaluation of the biological activity against herpes simplex virus type 1 (HSV-1) of *Cedruslibani* A. Rich. Phytomedicine, 15: 79-83.

[12] M'Barek LA, Ait Mouse H, Jaafari A, Aboufatima R, Benharref A, Kamal M, Benard J, El Abbadi N, Ben salah M, Gamouh A (2007). Cytotoxic effect of essential oil of thyme (*Thymus broussonettii*) on the IGR-OV1 tumor cells resistant to chemotherapy. Braz. J. Med. Biol. Res., 40: 1537-1544.

[13] Zarai Z, Kadri A, Chobba IB, Mansour RB, Bekir A, Mejdoub H, Gharsallah N (2011). The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of *Marrubium vulgare* L. Essential oil grown in Tunisia. Lipids Health Dis., 10: 161.

[14] Alviano DS, Alviano CS (2009). Plant extracts: Search for new alternatives to treat microbial diseases. Curr. Pharm. Biotechnol., 10: 106-121.

[15] Mazzant G, Battinelli L, Salvator G (1998). Antibacterial properties of the linalool rich essential oil of *Hyssopous officinalis* L. var. decumbens (Lamiaceae). FlavourFrag. J., 13: 298.

[16] da Silva AR, Lopes PM, de Azevedo MB, Costa DM, Alviano CS, Alviano DS (2012). Biological activities of α-pinene and β-pineneenantiomers. Molecules, 17: 6305-6316.

[17] Tabanca N, Demirci B, Crockett SL, Baser KHC, Wedge DE (2007). Chemical composition and antifungal activity of *Arnica longifolia*, *Aster hesperius*, and *Chrysothamnus nauseosus* essential oils. J. Agric. Food Chem., 55: 8430-8435.

[18] Yang Z, Wu N, Zu Y, Fu Y (2011). Comparative anti infectious bronchitis virus (IBV) activity of (−)-pinene: Effect on nucleocapsid (N) protein. Molecules, 16: 1044–1054.

[19] Garg SN, Naqvi A, Singh A, Ram A, Kumar S (1999). Composition of essential oil from an annual crop of *Hyssopus officinalis* grown in Indian plains. Flavour Frag. J., 14: 170-172.

[20] De Martino L, De Feo V, Nazzaro F (2009). Chemical composition and in vitro antimicrobial and mutagenic activities of seven Lamiaceae essential oils. Molecules, 14: 4213-4230.

[21] Svoboda KP, Galambosi B, Deans SG, Hethelyi E (1993). Agronomical and biochemical investigation of *Hyssopus officinalis* L. from various geographical sources. Acta Hort. 344: 434-443.

[22] Veres K, Varga E, Dobos A, Hajdn Z, Mathe I, Pluhar Z, Nemeth, E, Bernath J (1997). Investigation of the composition of essential oils of *Hyssopus officinalis* L. populations, 217-220 pp. In: Ch. Franz, A. Mathe, G. Buchbaner (Eds), Essential Oils: Basic and Applied Research, Allured Publishing Corporation, Carol Stream, IL, pp. 217-220.

[23] Vallejo M, Herraiz J, Perez-Alonso M, Velasco Negueruela A (1995). Volatile oil of *Hyssopus officinalis* L. from Spain. J. Essent. Oil Res. 7: 567-568.

[24] Jankovsky M, Land T (2002). Genus *Hyssopus* L.-Recent Knowledge. Hort. Sci., (Paragues) 29: 119-123.

[25] Mitic V, Dordevic S (2000). Essential oil composition of *Hyssopus officinalis* L. cultivated in Serbia. Facta Universitatis, Physics Chemistry and Technology, 2: 105-108.

[26] Gorunovic M, Bogavac P, Chalchat J, Chabardi J (1995). Essential oil of *Hyssopus officinalis* L. Lamiaceae of Montenegro origin. J. Essent. Oil Res., 7: 39-43.

[27] Wang N, Yang XW (2010). Two new flavonoid glycosides from the whole herbs of *Hyssopus officinalis*. J. Asian Natural. Prod. Res., 12:1044-1050.

[28] Said-Al Ahl HAH, Abbas ZK., Sabra AS., Tkachenko KG (2015). Essential oil composition of *Hyssopus officinalis* L. cultivated in Egypt. International Journal of Plant Science and Ecology, 1(2): 49-53.

[29] Marin FR, Ortuño A, Benavente-Garcia O, Del Rio JA (1998). Distribution of flavone glycoside diosmin in *Hyssopus officinalis* plants: Changes during growth. Planta Med., 64: 181-182.

[30] Marino M, Bersani C, Comi G (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and compositae. Int. J. Food Microbiol., 67: 187–195.

[31] Hilal SH, El-Alfy TS, El-Sherei MM (1979). Study of the flavonoid content of *Hyssopus officinalis* L. Egyptian J. Pharm. Sci., 20: 271-278.

[32] Murakami Y, Omoto T, Asai I, Shimomura K, Yoshihira K, Ishimaru K (1998). Rosmarinic acid and related phenolics in transformed root cultures of *Hyssopus officinalis*. Plant Cell, Tissue and Organ Culture, 53: 75-78.

[33] Varga E, Hajdu Z, Veres K, Mathe I, Nemeth E, Pluhar Z, Bernath J (1998a). Investigation of variation of the production of biological and chemical compounds of *Hyssopus officinalis* L. Acta. Pharm. Hung., 68: 183-188.

[34] Kochan E, Wysokinska H, Chmiel A, Grabias B (1999). Rosmarinic acid and other phenolic acids in hairy roots of *Hyssopus officinalis*. Biosciences, 54: 11-16.

[35] Zgorka G, Głowniak K (2001). Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. J. Pharm. Biomed. Analysis, 26: 79-87.

[36] Benedec D, Oniga I, Tipercius B, Popescu H (2002). Preliminary research of some polyphenolic compounds from *Hyssopus officinalis* L. (Lamiaceae). Farmacia, 50: 54-57.

[37] Proestos C, Chorianopoulos N, Nychas GJ, Komaitis EM (2005). RPHPL Canalysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. J. Agric. Food Chem., 53: 1190-1195.

[38] Recio MC, Giner RM, Manez S, Rios JL (1995), Structural requirements for the anti-inflammatory activity of natural triterpenoids. Planta Med., 61: 182-185.

[39] Cantrell CL, Franzblau SG, Fischer NH (2001), Antimycobacterial plant terpenoids. Planta Med., 67: 685-694.

[40] Ohigashi H, Takamura H, Koshimizu K, Tokuda H, Ito Y (1986). Search for possible antitumor pro moters by inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation, ursolic acid and oleanolic acid from an anti-inflammatory Chinese medicinal plant, *Glechoma hederacea* L. Cancer Lett., 30, 143-151.

[41] Rios MR, Gonzales-Morales A, Villarreal ML (2001), Sterols, triterpenes, bioflavonoids of *Viburnum jucundum*, and cytotoxic activity of ursolic acid. Planta Med., 67: 683-684.

[42] Macato SB, Sucharita S (1997), Advances in triterpenoid research 1990-1994. Phytochemistry, 44: 1185-1236.

[43] Tokuda H, Onigashi H, Koshimizu H (1986). Inhibitory effects of ursolic and oleanolic acid on skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. Cancer Lett., 33, 279-285.

[44] Liu J (1995), Pharmacology of oleanolic acid and ursolic acid. J. Ethnopharmacology 49, 57-68.

[45] Skrzypek Z, Wysokinska H (2003). Sterols and triterpenes in cell Culture of *Hyssopus officinalis* L. Z. Naturforsch., 58c, 308-312.

[46] Ali N, Mekuria AH, Requena JM, Engwerda C (2012). Immunity to visceral leishmaniasis, J. Trop. Med., (2012):780-809.

[47] World Health Organization (2010). Control of the leishmaniases. Geneva: World Health Organization; 2010.

[48] Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, den Boer M (2012). Leishmaniasis worldwide and global estimates of its incidence. PLoS One, 7 (5): e35671.

[49] Arevalo J, Ramirez L, Adaui V, Zimic M, Tulliano G, Miranda-Verástegui C, Lazo M, Loayza-Muro R, De Doncker S, Maurer A, Chappuis F, Dujardin JC, Llanos-Cuentas A (2007). Influence of *Leishmania* (*Viannia*) species on the response to antimonial treatment in patients with American tegumentary leishmaniasis. J. Infect Dis., 195 (12): 1846–1851.

[50] Accioly MP, Bevilaqua CM, Rondon FC, de Morais SM, Machado LK, Almeida CA, de Andrade HFJ, Cardoso RP (2012). Leishmanicidal activity in vitro of *Musa paradisiaca* L. and *Spondia smombin* L. fractions. Vet. Parasitol., 187 (1–2): 79–84.

[51] Date A, Joshi MD, Patravale VB (2007). Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles, Adv. Drug Deliv. Rev., 59 (6): 505–521.

[52] Corral MJ, Benito-Peña E, Jiménez-Antón MD, Cuevas L, Moreno-Bondi MC, Alunda JM (2016). Allicin induces calcium and mitochondrial dysregulation causing necrotic death in *leishmania*, PLoSNegl. Trop. Dis., 10 (3): e0004525.

[53] Markel L, Voge M (2006). Medical Parasitology, Edn 9, Saunders Elsevier Press, USA.

[54] Hepburn NC (2001). Management of cutaneous leishmaniasis. Curr. Opin. Infect Dis., 14(2):151-154.

[55] Akhlaghi L, Yeganeh M, Maleki F, Golestani M, Noori M, Ghelman M, Tabatabaie F (2014). Anti-leishmanial activity of *Hyssopus officinalis*, *Tussilage farfara*, *Carum copticum* extracts in mice infected with *Leishmania major*. International Journal of Herbal Medicine, 2(2): 142-145.

[56] Iwu MM, Jackson JE, Schuster BG (1994). Medicinal plants in the fight against leishmaniasis. Parasitol. Today, 10:65.

[57] Lerner EA, Grevelink SA (1996). Leishmaniasis. In: Arndt KA, Leboil PE, Robinson JK, editors. Cutaneous medicine and surgery. Philadelphia: W. B. Saunders, 1163-70.

[58] Maleki F, Zarebavani M, Mohebali M, Dayer S, Hajialiani F, Tabatabaie F (2017). In vitro and *in vivo* susceptibility of *Leishmania major* to some medicinal plants. Asian Pacific Journal of Tropical Biomedicine, 7 (1): 37–42.

[59] Rosa MdS, Mendonça-Filho RR, Bizzo HR, Rodrigues Id, Maria R, Soares A, Souto-Padrón T, Alviano CS, Lopes AHCS (2003). Antileishmanial activity of a linalool-rich essential oil from *Croton cajucara*. Antimicrob. Agents Chemother., 47 (6): 1895-1901

[60] Alexander J, Satoskar AR, Russel DG (1999). Leishmania species: models of intracellular parasitism. J. Cell Sci., 112: 2993-3002.

[61] Hall LR, Titus RG (1995). Sand fly saliva selectively modulates macrophage functions that inhibit killing of *Leishmania major* and nitric oxide production. J. Immunol., 155:3501-3506.

[62] Liew FY, O'Donnel CA (1993). Immunology of leishmaniasis. Adv. Parasitol., 32:161-259.

[63] Desjeux P (1991). Information on the epidemiology and control of the leishmaniases by country and territory. Report no. 27. World Health Organization, Geneva, Switzerland.

[64] Lopes AHCS, Dutra PML, Rodrigues CO, Soares MJ, Angluster J, Cordeiro RSB (1997). Effect of platelet-activating factor on the process of cellular differentiation of *Herpetomonasmu scarum*. J. Eukaryot. Microbiol., 44:321-325.

[65] Alvar J, Cañavate C, Gutiérrez-Solar B, Jiménez M, Laguna F, López-Vélez R, Molina R, Moreno J (1997). *Leishmania* and human immunodeficiency virus co-infection: the first 10 years. Clin. Microbiol. Rev., 10:298-319.

[66] Olliaro PL, Bryceson ADM (1993). Practical progress and new drugs for changing patterns of leishmaniasis. Parasitol. Today, 9:323-328.

[67] Fournet A, Hocquemiller R, Roblot F, Cavé A, Richomme P, Bruneton J (1993). Les chimanines, nouvellesquinoléinessubstituées en 2 isoléesd′uneplanteboliviennеantiparasitaire: *Galipea longiflora*. J. Nat. Prod., 56:1547-1552.

[68] Fournet A, Hocquemiller R, Gantier JC (1995). Combattre la leishmaniose. Recherche, 26:424-429.

[69] Croft SL, Yardley V (2002). Chemotherapy of leishmaniasis. Curr. Pharm. Des., 8:319-342.

[70] Rates SMK (2001). Plants as source of drugs. Toxicon, 39:603-613.