NPC Natural Product Communications

2011
Vol. 6
No. 11
1697 - 1700

In vitro Antiprotozoal Activity of Extracts of five Turkish Lamiaceae Species

Hasan Kirmizibekmez[a], Irem Atay[a], Marcel Kaiser[b], Erdem Yesilada[a] and Deniz Tasdemir[c,*]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, University of Yeditepe, 34755 Kayisdagi, Istanbul, Turkey*

[b]*Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Socinstr. 57, CH-4002, Basel, Switzerland*

[c]*Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, United Kingdom*

deniz.tasdemir@pharmacy.ac.uk

Received: June 14th, 2011; Accepted: August 22nd, 2011

The *in vitro* antiprotozoal activities of crude methanolic extracts from the aerial parts of five Lamiaceae plants (*Salvia tomentosa*, *S. sclarea*, *S. dichroantha*, *Nepeta nuda* subsp. *nuda* and *Marrubium astracanicum* subsp. *macrodon*) were evaluated against four parasitic protozoa, i.e. *Trypanosoma brucei rhodesiense*, *T. cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. The cytotoxic potentials of the extracts on L6 cells were also evaluated. Melarsoprol, benznidazole, miltefosine, chloroquine and podophyllotoxin were used as reference drugs. All crude MeOH extracts showed antiprotozoal potential against at least three parasites, so they were dispersed in water and partitioned against *n*-hexane and chloroform to yield three subextracts that were screened in the same test systems. The *n*-hexane extract of *N. nuda* was the most active against *T. brucei rhodesiense* while the $CHCl_3$ extracts of *S. tomentosa* and *S. dichroantha* showed significant activity against *L. donovani*. All organic extracts displayed *in vitro* antimalarial and moderate trypanocidal activities against *T. cruzi* with the *n*-hexane extract of *S. sclarea* being the most active against the latter. The extracts displayed low or no cytotoxicity towards mammalian L6 cells.

Keywords: Lamiaceae, *Nepeta*, *Salvia*, *Marrubium*, Antiprotozoal activity.

Tropical neglected diseases, i.e. African trypanosomiasis, Chagas disease, visceral leishmaniasis and malaria continue to be major health problems that affect millions of people worldwide. *T. brucei rhodesiense*, *T. cruzi*, *Leishmania donovani*, *Plasmodium falciparum* are the causative agents of these illnesses respectively [1a]. African trypanosomiasis, more commonly known as sleeping sickness, is caused by single-celled protozoa from the *Trypanosoma* genus. One of the two major species that infect humans is *Trypanosoma brucei rhodesiense*. The disease is limited to Africa, and outbreaks tend to affect isolated rural areas. Around 12,000 new cases were reported in 2006 [1b]. Chagas' disease (American trypanasomiasis) is an indigenous disease of 22 countries in the Western Hemisphere caused by the protozoa *Trypanosoma cruzi* [1c,2]. It has been estimated that there are 15-17 million people infected by *T. cruzi* in Latin America and 90-100 million are exposed to infection [3]. Leishmaniasis is caused by obligate intracellular and kinetoplastid protozoa of the genus *Leishmania* that is endemic to 88 countries with 350 million people at risk of infection [4a]. Parallel infections with diseases such as malaria and pneumonia increases the fatality of the illness, as do parallel infections of AIDS and the development of drug-resistance by the parasites [4b]. Visceral leishmaniasis, also known as kala-azar, is caused by *Leishmania donovani* [4c]. Malaria infects 300-500 million people and kills over 1 million people annually. Human malaria is endemic to 90 countries and is caused by the protozoa *Plasmodium falciparum* [4d].

The Lamiaceae family of mostly medicinal and aromatic plants is represented by around 3000 species in 250 genera worldwide. Turkish flora contains 558 species and 45 genera belonging to the Lamiaceae, almost half of which are endemic [5a]. *Salvia* (sage), the largest genus with 900 species worldwide [5b] and 90 in Turkey is commonly known as "adaçayı" [5a]. It is traditionally used as herbal tea with carminative, spasmolytic, diuretic, antiseptic and wound healing properties, as well as against rheumatic and abdominal pains, cold and cough [5c-5e]. In South African traditional medicine, some *Salvia* species are reported to treat fever and febrile attacks [6]. *In vitro* antiplasmodial activities of several *Salvia* species against *Plasmodium falciparum* were also reported [7,8a]. The genus *Nepeta* is represented by 33 species in Turkish flora and half of these species are endemic [5a] and used in traditional medicine to treat bronchitis [8b]. The genus *Marrubium* consists of perennial herbs of which 18 species grow in Turkey [5a]. Some species are used to treat bronchitis, cough, fever and

asthma in folk medicine [9,10a]. Promising anti-microbial activity of *M. vulgare* extracts against Gram-positive bacteria was reported [10b]. As part of the systematic survey of the antiprotozoal activities of Turkish medicinal plants [10c,11a,11b] this study describes the *in vitro* antimalarial, trypanocidal, leishmanicidal and the cytotoxic activities of extracts of five Lamiaceae herbs of the genera *Salvia*, *Nepeta* and *Marrubium* native to Turkey.

In order to evaluate their *in vitro* antiprotozoal activity, the crude methanolic extracts of *Salvia tomentosa*, *S. sclarea*, *S. dichroantha*, *Nepeta nuda* subsp. *nuda* and *Marrubium astracanicum* subsp. *Macrodon*) were tested *in vitro* against four parasitic protozoa, *Trypanosoma brucei rhodesiense*, *T. cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. The extracts were simultaneously tested on rat skeletal myoblasts (L6 cells) in order to determine their selectivity. Table 1 shows the results. All crude MeOH extracts displayed antiprotozoal potential against at least three parasites without any cytotoxicity. This includes trypanocidal activity against *T. brucei rhodesiense* with the extracts of *N. nuda* (Nn-MeOH), *S. dichroantha* (Sd-MeOH) and *S. sclarea* (Ss-MeOH) being the most potent, antileishmanial activity against *L. donovani* amastigotes, with Sd-MeOH showing the highest activity, and antiplasmodial activity with the extracts of *Marrubium astracanicum* subsp. *macrodon* (Ma-MeOH) and Ss-MeOH displaying the highest activity. On the contrary, all MeOH extracts were inactive against *T. cruzi* except for *S. sclarea*, which had mild activity. Remarkably, only the *S. sclarea* MeOH extract showed a marginal cytotoxicity against mammalian cells.

The crude MeOH extracts were dispersed in H_2O and partitioned first against *n*-hexane, and then against $CHCl_3$. All three subextracts (*n*-hexane, $CHCl_3$ and H_2O) were screened as shown in Table 1. All organic extracts showed antiprotozoal activity in varying degrees. Against *T. brucei rhodesiense,* the *n*-hexane extract of *N. nuda* (Nn-Hexane) showed very significant activity followed by the *n*-hexane (St-Hexane) and $CHCl_3$ extracts (St-$CHCl_3$) of *S. tomentosa* and the *n*-hexane extract of *S. sclarea* (Ss-Hexane). Interestingly, all aqueous extracts displayed trypanocidal potential with the water subextract of *S. dichroantha* (Sd-H_2O) being the most active and the water subextract of *Marrubium astracanicum* subsp. *macrodon* (Ma-H_2O) being the least active. Against the American trypanosomes (*T. cruzi*), all organic extracts were only moderately active whereas all aqueous subextracts were inactive.

All *n*-hexane and chloroform extracts had antileishmanial activity. The $CHCl_3$- and *n*-hexane-solubles of *S. tomentosa* (St-$CHCl_3$ and St-Hexane) and the $CHCl_3$-solubles of *S. dichroantha* (Sd-$CHCl_3$) exhibited the most significant activity against *L. donovani*. A similar bioactivity profile was apparent against the drug resistant malaria parasite *P. falciparum,* where all organic extracts were quite active and H_2O extracts were not. The $CHCl_3$ extracts of *S. sclarea* and *N. nuda* were the most potent. When tested against mammalian L6 cells, only the organic extracts of *S. tomentosa*, the *n*-hexane extract of *S. sclarea* and the $CHCl_3$ extract of *N. nuda* showed cytotoxicity. The remaining extracts had marginal or no toxicity at the highest test concentrations (IC_{50}s>90 μg/mL).

Table 1: Trypanocidal, leismanicidal, antiplasmodial and cytotoxic activities of the solvent extracts of *Salvia tomentosa* (St), *S. sclarea* (Ss), *S. dichroantha* (Sd), *Nepeta nuda* subsp. *nuda* (Nn) and *Marrubium astracanicum* subsp. *macrodon* (Ma). All IC_{50} values are in μg/mL.

Extract	*T. b. rhodesiense*	*T. cruzi*	*L. donovani*	*P. falciparum*	Cytotoxicity (L6 cells)
St-MeOH	3.64	>90	14.92	9.94	90
St-Hexane	1.24	28.46	2.49	3.47	26.24
St-$CHCl_3$	2.33	35.72	1.81	3.14	31.8
St-H_2O	10.96	>90	>90	>20	>90
Ss-MeOH	6.44	56.82	12.95	6.60	87.6
Ss- Hexane	2.40	18.17	5.25	3.78	18.3
Ss-$CHCl_3$	4.40	52.51	8.31	2.54	83.9
Ss-H_2O	10.31	>90	47.88	>20	>90
Sd-MeOH	3.58	>90	4.93	8.85	>90
Sd-Hexane	3.50	41.85	3.48	4.17	>90
Sd-$CHCl_3$	4.40	48.99	2.31	3.72	84.8
Sd-H_2O	7.77	>90	>90	>20	>90
Nn-MeOH	3.43	>90	15.26	11.31	>90
Nn-Hexane	0.62	46.77	7.73	3.37	85
Nn-$CHCl_3$	3.57	55.98	4.08	2.95	46.2
Nn-H_2O	10.64	>90	>90	>20	>90
Ma-MeOH	4.69	>90	15.81	6.39	>90
Ma-Hexane	3.29	58.33	9.39	4.64	88.3
Ma-$CHCl_3$	8.56	49.56	7.17	3.36	>90
Ma-H_2O	35.90	>90	>90	>20	>90
Reference drug	0.003[a]	0.359[b]	0.2[c]	0.056[d]	0.004[e]

Reference compounds: [a]Melarsoprol, [b]benznidazole, [c]miltefosine, [d]chloroquine, [e]podophyllotoxin

In summary, the organic extracts were generally the most potent probably due to enrichment of more lipophilic compounds. Overall *T. brucei rhodesiense* appears to be the most susceptible protozoan species and with the subμg/mL level activity and almost no toxicity on mammalian cells, the hexane extract of *N. nuda* is very promising. With a selectivity index (=therapeutic index, calculated by dividing the IC_{50} value against L6 cells / the IC_{50} value against parasite) of 137, this extract clearly merits further activity-guided isolation studies which are currently underway in our laboratory. In general, the hexane subextracts had a higher activity than the $CHCl_3$ extracts against both *Trypanosoma* species. The organic subextracts, in particular the $CHCl_3$ extracts proved to have good leishmanicidal and antiplasmodial properties. All three *Salvia* species investigated have remarkable antiprotozoal potential with S. *dichroantha* the most promising in terms of low toxicity profile. Despite the fact that only one *Marrubium* species was studied, *M. astracanicum* subsp. *macrodon* seems to be worthy of further chemical and biological studies, particularly for antiplasmodial activity.

Several species of *Salvia* have been shown to exert antimalarial activity [11c]. The lipophilic extracts of *Salvia* species are rich in diterpenes and triterpenes [12a], several of which possess antileishmanial, [12b] trypanocidal [13a] and antimalarial [7] activities. Monoterpenoid nepetalactones, sterols and triterpenes have previously been isolated from the *n*-hexane extract of *Nepeta nuda* subsp. *albiflora* [13b], which may be responsible for the

antiparasitic activity. Likewise, the labdane diterpenoids and methoxylated flavones [9,13c] reported from the lipophilic extracts of *Marrubium* species could be responsible for the antiprotozoal activities of *M. astracanicum* subsp. *macrodon*.

Experimental

Plant material: *Salvia tomentosa*, *S. sclarea*, *S. dichroantha*, *Nepeta nuda* subsp. *nuda* and *Marrubium astracanicum* subsp. *macrodon* were collected in July 2008 from Eskişehir, Turkey, and identified by Dr. Akaydin. The voucher specimens (Akaydın 10934, 11086, 10539, 11158 and 11151, respectively) deposited at the Herbarium of the Faculty of Education, Hacettepe University, Ankara, Turkey.

Preparation of the extracts: The air-dried and powdered aerial parts of each plant (20 g) were macerated overnight in MeOH (200 mL) and filtered. The residues were further extracted with MeOH (200 mL) at 45°C for 4 hours. After filtration and combination with the initial MeOH macerates, the crude methanolic extracts were evaporated and dried under *vacuum*. The extract yields were 15% for *S. tomentosa* and *S. sclarea*, 12% for *S. dichroantha*, 11% *N. nuda* subsp. *nuda*, and 10% for *M. astracanicum* subsp. *macrodon*. The crude MeOH extracts were dispersed in H_2O (5 mL) and partitioned against *n*-hexane (3 x 5 mL) and $CHCl_3$ (3 x 5 mL), respectively. The organic solvents were evaporated to dryness under reduced pressure while the H_2O extracts were freeze-dried. All the extracts were stored at -20°C until use.

***In vitro* assay for *Plasmodium falciparum*:** *In vitro* activity against erythrocytic stages of *P. falciparum* was determined by a modified [^{3}H]-hypoxanthine incorporation assay, using the chloroquine- and pyrimethamine-resistant K1 strain and the standard drug chloroquine. Briefly, parasite cultures incubated in RPMI 1640 medium with 5% Albumax (without hypoxanthine) were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37°C in a reduced oxygen atmosphere, 0.5 µCi ^{3}H-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a Betaplate^{TM} liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. IC_{50} values were calculated from the sigmoidal inhibition curves using Microsoft Excel.

***In vitro* assay for *Trypanosoma brucei rhodesiense*:** *T. b. rhodesiense*, STIB 900 strain, and the standard drug, melarsoprol, were used for the assay. This stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions. Minimum Essential Medium (50 µL) supplemented with 25 mM HEPES, 1g/l additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1mM Na-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of seven 3-fold dilution steps were prepared. Then 10^4 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 µL was added to each well and the plate incubated at 37°C under a 5% CO_2 atmosphere for 72 h. 10 µL Alamar Blue (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2-4 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA).

***In vitro* assay for *Trypanosoma cruzi*:** Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 µL RPMI 1640 medium with 10% FBS and 2 mM l-glutamine. After 24 h the medium was removed and replaced by 100 µL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene. After 48 h, the medium was removed from the wells and replaced by 100 µl fresh medium with or without a serial drug dilution. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then the substrate CPRG/Nonidet (50 µL) was added to all wells. A color reaction developed within 2-6 h and could be read photometrically at 540 nm. Data were transferred into the graphic programme Softmax Pro (Molecular Devices), which calculated IC_{50} values. Benznidazole was the standard drug used.

***In vitro* assay for *Leishmania donovani*:** Amastigotes of *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37°C in SM medium at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO_2 in air. One hundred microlitres of culture medium with 10^5 amastigotes from axenic culture with or without a serial drug dilution were seeded in 96-well microtitre plates. Serial drug dilutions were prepared. After 72 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. Alamar Blue (10 µL; 12.5 mg resazurin dissolved in 100 ml distilled water) were then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (= inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. From the sigmoidal inhibition curves the IC_{50} values were calculated. Miltefosine was used as a reference drug.

***In vitro* assay for cytotoxicity:** Assays were performed in 96-well microtiter plates, each well containing 100 µL of

RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4 x 10^4 L6 cells (a primary cell line derived from rat skeletal myoblasts). Serial drug dilutions of seven 3-fold dilution steps covering a range from 90 to 0.123 μg/mL were prepared. After 72 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. Alamar Blue (10 μL, 12.5 mg resazurin dissolved in 100 ml double-distilled water) was then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analysed using the microplate reader software Softmax Pro. Podophyllotoxin was the standard drug used.

Acknowledgments - The authors wish to thank Dr. Galip Akaydın (Hacettepe University, Department of Biology Education, Ankara, Turkey) for authentification of the plant materials.

References

[1] (a) World Health Organization. (**2006**) Neglected tropical diseases, hidden successes, emerging opportunities web page. Available at http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_2006.2_eng.pdf. 2006; (b) Nimmo C. (**2010**) Time to put out the lights on sleeping sickness? *Travel Medicine and Infectious Disease*, ***8***, 263-268; (c) Schmunis GA, Yadon ZE. (**2010**) Chagas disease: A Latin American health problem becoming aworld health problem. *Acta Tropica*, ***115***, 14–21.

[2] Abad-Francha F, Santos WS, Schofield CJ. (**2010**) Research needs for Chagas disease prevention. *Acta Tropica*, ***115***, 44–54.

[3] Coura JR, Borges-Pereira J. (**2010**) Chagas disease: 100 years after its discovery. A systematic review. *Acta Tropica*, ***115***, 5–13.

[4] (a) Ngurea PK, Kimutai A, Ng'ang'a ZW., Rukunga G, Tonui WK. (**2009**) A review of Leishmaniasis in Eastern Africa. *Journal of Nanjing Medical University*, ***23***, 79-86; (b) Rocha LG. Almeida JRGS, Macedo RO, Barbosa-Filho JM. (**2005**) A review of natural products with antileishmanial activity. *Phytomedicine*, ***12***, 514-535; (c) Boelaert M, Criel B, Leeuwenburg J. Van Damme W, Le Ray D, Van der Stuyft P. (**2000**) Visceral leishmaniasis control: a public health persptictive. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, ***94***, 465 -471; (d) Da Silva Filho AA, Resende DO, Fukui MJ, Santos FFP, Pauletti M, Cunha, WR, Silva, MLA, Gregório LE, Bastos, JK, Nanayakkara NPD. (**2009**). *In vitro* antileishmanial, antiplasmodial and cytotoxic activities of phenolics and triterpenoids from *Baccharis dracunculifolia* D.C. (Asteraceae). *Fitoterapia*, ***80***, 478-482.

[5] (a) Davis PH. (**1982**) *Flora of Turkey and East Aegean Islands*. Vol. *7*, Edinburg: University Press; (b) Cioffi G, Bader A, Malafronte A, Piaz FD, De Tomassi N. (**2008**) Secondary metabolites from the aerial parts of *Salvia palaestina* Bentham. *Phytochemistry*, ***69***, 1005-1012; (c) Baytop T. (**1999**) Therapy with Medicinal Plants in Turkey (past and present), 2nd edn. Istanbul Nobel Tıp Kitapevleri; (d) Honda G, Yesilada E, Tabata M, Sezik E, Fujita T, Takeda Y, Takaishi Y, Tanaka T. (**1996**) Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kutahya, Denizli, Mugla provinces. *Journal of Ethnopharmacology*, ***53***, 75-87; (e) Simsek I, Aytekin F, Yesilada E, Yildirimli S. (**2004**) An Ethnobotanical Survey of the Beypazarı, Ayas and Güdül District towns of Ankara province (Turkey). *Economic Botany*, ***58***, 705-720.

[6] Kamatou GPP, Makunga NP, Ramogola WPN, Viljoen AM. (**2008**) South African *Salvia* species: A review of biological activities and phytochemistry. *Journal of Ethnopharmacology*, ***119***, 664-672.

[7] Kamatou GPP, Van Zyl RL, Davids H, van Vuuren SF, Lourens AC, Viljoen AM. (**2008**) The antimalarial and anticancer activities of selected South African *Salvia* species and isolated compounds from *S. radula*. *South African Journal of Botany*, ***74***, 238-243.

[8] (a) Clarkson C, Maharaj VJ, Crough NR. (**2004**) *In vitro* antiplasmodial activity of medicinal plants native to or neutralized in South Africa. *Journal of Ethnopharmacology*, ***92***, 177-191; (b) Yesilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T, Takeda Y. (**1995**) Traditional Medicine in Turkey V. Folk medicine in the inner Taurus Mountains, *Journal of Ethnopharmacology*, ***46***, 133-152.

[9] Argyropoulou C, Karioti A, Skaltsa H. (**2009**) Labdane diterpenes from *Marrubium thessalum*. *Phytochemistry*, ***70***, 635-640.

[10] (a) Esmaeili S, Naghibi F, Mosaddegh M, Sahranavard S, Ghafari S, Abdullah NR. (**2009**) Screening of antiplasmodial properties among some traditionally used Iranian plants. *Journal of Ethnopharmacology*, ***121***, 400-404; (b) Al-Bakri AG, Afifi FU. (**2007**) Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. *Journal of Microbioogicall Methods*, ***68***, 19-25; (c) Kirmizibekmez H, Perozzo R, Brun R, Linden A, Rüedi P, Dönmez AA, Çalıs I, Tasdemir D. (**2004**). Inhibiting activities of the secondary metabolites of *Phlomis brunneogaleata* against parasitic protozoa and plasmodial enoyl-ACP reductase, acrucial enzyme in fatty acid biosynthesis. *Planta Medica*, ***70***, 711–717.

[11] (a) Tasdemir D, Güner ND, Perozzo R, Brun **R**, Dönmez AA, Çalıs I, Rüedi P. (**2005**) Antiprotozoal and plasmodial FabI enzyme inhibiting metabolites of *Scrophularia lepidota* roots. *Phytochemistry*, ***66***, 355-362; (b) Tasdemir D, Brun R, Franzblau, SG, Sezgin Y, Çalıs I. (**2008**) Evaluation of antiprotozoal and antimycobacterial activities of the resin glycosides and the other metabolites of *Scrophularia cryptophila*. *Phytomedicine*, ***15***, 209-215; (c) Kamatou GPP, Viljoen AM, Gono-Bwalya AB, van Zyl RL, van Vuuren SF, Lourens ACU, Baser, KHC, Demirci B, Lindsey KL, van Staden J, Steenkamp P. (**2005**) The *in vitro* pharmacological activities and a chemical investigation of three South African *Salvia* species. *Journal of Ethnopharmacology*, ***102***, 382-390.

[12] (a) Ulubelen A, Topcu G. (**1998**) Chemical and biological investigations of *Salvia* species growing in Turkey. *Studies in Natural Products Chemistry*, ***20***, 659-718; (b) Tan N, Kaloga M, Radtke OA, Kiderlen AF, Öksüz S, Ulubelen A, Kolodziej H. (**2002**) Abietane diterpenoids and triterpenoic acids from *Salvia cilicica* and their antileishmanial activities. *Phytochemistry*, ***61***, 881-884.

[13] (a) Sanchez AM, Jimenez-Ortiz V, Sartor T, Tonn CE, García EE, Nieto M, Burgos MH, Sosa MA. (**2006**) A novel icetexane diterpene, 5-epi-icetexone from *Salvia gilliessi* is active against *Trypanosoma cruzi*. *Acta Tropica*, ***98***, 118-124; (b) Kökdil G, Yalçın SM, Topcu G. (**1999**) Nepetalactones and other constituents of *Nepeta nuda* ssp. *albiflora*. *Turkish Journal of Chemistry*, ***23***, 99-104; (c) Hennebelle T, Sahpaz S, Skaltsounis AL, Bailleul F. (**2007**) Phenolic compounds and diterpenoids from *Marrubium peregrinum*. *Biochemical Systematics and Ecology*, ***35***, 624-626.