Asian Journal of Chemistry; Vol. 23, No. 11 (2011), 4810-4814

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Isolation of Artediffusin (Tehranolide) as a New Antimalarial Agent

ABDOLHOSSEIN RUSTAIYAN[1,*], HOSSEIN NAHREVANIAN[2] and MASOUD KAZEMI[3]

[1]Department of Chemistry, Science and Research Branch, Islamic Azad University, P.O. Box 14515-775, Tehran, Iran
[2]Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran
[3]Faculty of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran

*Corresponding author: E-mail: arustaiyan@yahoo.it

(*Received*: 22 September 2010; *Accepted*: 11 July 2011) AJC-10156

A few alternative drugs are under development, necessitating urgent efforts to identify new classes of antimalarial agents. There is a need to find new, effective and affordable remedies for malaria, including those derived from plants. The clinical utility of the Chinese discovery of artemisinin from the herb *Artemisia annua* has stimulated much interest in traditional plants as potential sources of new antimalarial drugs. The emergence of drug-resistant strains of *Plasmodium falciparum* has resulted in an urgent need to develop new antimalarial chemotherapeutic agents. The rich plant diversity and long history of traditional medicine in Iran warrants investigation and may be a valuable source of novel compounds. In this study, the antimalarial activity of artediffusin (tehranolide), a sesquiterpene lactone with an endoperoxide group, on plasmodium berghei *in vivo* on the mice model of malaria was investigated. It demonstrates that artediffusin which has been isolated from *Artemisia diffusa* inhibit the growth of plasmodium berghei *in vivo* in NMRI mice.

Key Words: New antimalarial agent, *Artemisia diffusa*, *Plasmodium berghei*, Artediffusin (Tehranolide).

INTRODUCTION

Malaria is one of the most serious health problems in many parts of the world, particularly in Africa and Latin America with a high mortality rate. The situation is further complicated by the spread of drug-resistant parasites in many parts where *Plasmodium falciparum* is endemic. Despite significant progress in malaria control over the last few years, malaria-related morbidity and mortality are still considerable[1]. The malaria situation is aggravated by the appearance of strains of *Plasmodium falciparum* resistant to antimalarial drugs as well as by the resistance of vector Anopheles mosquitoes to DDT and other insecticides. Nearly 300-500 million people are infected by malaria and the incidence of this disease is dramatically increasing, since many strains of *Plasmodium falciparum*, the parasite responsible for the majority of fatal malaria infections, have become resistant to chloroquine and other traditional antimalarial drugs[2]. In Africa alone the disease is assumed to be responsible for the about 1.0-2.7 million deaths annually[3].

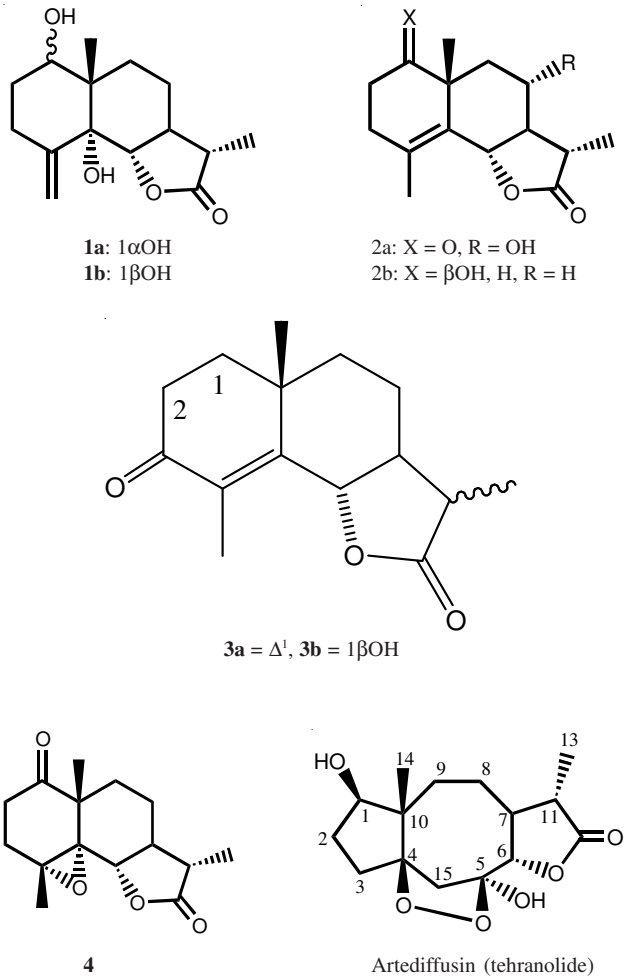
Malaria is a disease of poverty. Three-quarters of the population in rural Africa lives in extreme poverty, as defined by the family only having the income to buy enough food toward off starvation. The absence of vaccine and the emergence of drug-resistant strains render the eradication and the control of this disease nearly impossible.

These are the principal factors that contribute to the difficulty of the malaria control. Studies in a number of African countries have shown that the emergence of the chloroquine-resistant malaria parasites is associated with a two-fold increase in malaria deaths, but in one study in Mlomp Senegal, it was shown that malaria mortality in children under the age of four increased 11-fold within 6 years of the emergence of the chloroquine-resistance[4]. This fact provides the reasons for research for new antimalarial drugs. Hence, the search for new drugs against malaria has become a pressing global demand.

Fortunately, these strains are still susceptible to the artimisinin derivatives. Artemisinin was originally isolated from *Artemisia annua*, an herb used as an ancient Chinese herbal remedy. *A. annua* is an annual herb native to the northern part of Chahar and Suiyuan provinces in China, where it is called "Qing Hao" for more than 2000 years[5,6]. *A. annua* is presently being cultivated on a commercial scale in China and Vietnam for its antimalarial sesquiterpene lactone. The genus artemisia has always been of great botanical and pharmaceutical interest and is useful in traditional medicines for a treatment of the variety of diseases and complaints. This genus including some Iranian species has been studied chemically and present of monoterpenes[7], sesquiterpenes[8-10], especially sesquiterpene lactones[7,11-15] and essential oils[8,16-20] were reported. This genus is not very uniform and the chemistry is

somewhat diverse. However, most species contain sesquiterpene lactones, especially 11,13-dihydro derivatives.

The extract of the aerial parts of *A. diffusa* Krasch ex P. Poljakov collected in the Province of Khorassan (Iran) afforded, in addition to several eudesmanolides [1a,1b,2a, 2b,3a,3b,4] and a new type of sesquiterpene lactone artediffusin (tehranolide) (Fig. 1)[11], with an endoperoxide group that probably has the same effect as the antimalarial agent artemisinin. We reported antimalarial effect of the extract of *A. diffusa* against *P. berghei*. Since the endoperoxide group is an essential requirement for the antimalarial activity of artemisinin, we have presumed the antimalarial properties of the crude extract are attributed to artediffusin (tehranolide)[21]. We report here the *in vivo* laboratory evaluation of antimalarial effect of artediffusin (tehranolide), was done on *P. berghei* infected NMRI mice. The antimalarial effects of artediffusin in 27 mg/mL concentration, (high dose) were injected S.C. every day for 12 days in malaria mice. Three groups of mice (n = 5) were investigated for antimalarial efficacy, the degree of parasitaemia, assessment of pathology including body weight, physiological activities, hepatomegaly and splenomegaly. Parasitaemia was measured every day by counting Geimsa-stained blood smears which were taken from end tail cutting of infected mice[22].



1a: 1αOH
1b: 1βOH

2a: X = O, R = OH
2b: X = βOH, H, R = H

3a = Δ^1, **3b** = 1βOH

4

Artediffusin (tehranolide)

Fig. 1. Eudesmanolides and a new type of sesquiterpene lactone Artediffusin (Tehranolide) from *Artemisia difussa*

EXPERIMENTAL

Plants were identified and collected in September 2007 from their natural habitats in North East of Iran Province of Khorassan by Dr. V. Mozaffarian of the Research Institute of Forests and Rangelands (TARI). Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands, Tehran.

Extraction and isolation: Ground aerial parts (950 g) were extracted with Et_2O/MeOH/petrol (1:1:1) (2 × 8 L) at room temperature for 48 h. Evaporation at reduced pressure furnished (39.5 g) of crude extract was suspended in EtOH (680 mL), diluted with H_2O (550 mL) and extracted successively with hexane (2 × 700 mL) and $CHCl_3$ (2 × 500 mL). Evaporation of the $CHCl_3$ extract at reduced pressure furnished (15.5 g) of reside, which was column chromatographed over silica gel (430 mg, 70- 230 mesh) using $CHCl_3$ and increasing amounts of EtOAc (0-100 %) and EtOAc/MeOH (9:1) to afford 33 fractions. These were grouped according to their TLC profiles and monitored by IR spectroscopy. Only fractions showing γ-lactone absorption in the 1780-1765 cm^{-1} range were processed. Fractions 7-8 (250 mg) were reunited and rechromatographed on silica gel (230-400 mesh) to give (48 mg) 1b. Fractions 10-13 (260 mg) were combined and portion of (125 mg) was processed by HPLC using a C_{18} column (MeOH/H_2O 6:4) flow rate, 3 mL/min gave (38 mg) 2b (Rt 8.5 min) and 12 mg 4 (R_t 13 min). Fractions 16-20 (240 mg) were reunited and rechromatographed on silica gel (230- 400 mesh) using Et_2O/MeOH (9:1) to yield (20 mg) of 1a, (41 mg) 3a and (11 mg) 2a. Fractions 26-33 (260 mg) were combined and portion (130 mg) was processed by HPLC using a RP8 column (MeOH/H_2O 5.5:4.5) flow rate, 3 mL/min to give (15 mg) 3b (R_t 5.5 min) and (15 mg) artdiffusin (tehranolide) (R_t 4 min). Compounds were identified by comparing the 500 MHz ^{1}H NMR spectra with those of authentic material.

NMR spectra were recorded at 500 MHz, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X32 control. IR spectra were taken on a BOMEM Canada FT-IR, MB-100 spectrometer. Silica gel 60 (70-230 and 230-400 mesh) and TLC was performed with Kieselgel 60 F_{254} (Merck aluminium support plates) and spot were detected after spraying with a 15 % H_2SO_4 solution in MeOH. For separation of mixtures, a Knauer HPLC instrument with a 1001 pump detector was used and column C_{18} (120 mm × 8 mm ID) was employed.

Male outbred NMRI mice (supplied by the Karaj Laboratory Animal Unit, Pasteur Institute of Iran) were used in this study. The mice were housed at room temperature (20-23 ºC) on a 12 h light and 12 h dark cycle, with unlimited access to food and tap water. Experiments with animals were done according to the ethical standards formulated in the Declaration of Helsinki and measures taken to protect animals from pain or discomfort. It has been approved by institutional ethical review board (Ethical Committee of the Pasteur Institute of Iran), in which the antimalarial test was done.

Malaria parasites: *Plasmodium berghei* NY kindly donated by Dr. M.J. Dascombe from the School of Life Sciences,

University of Manchester, UK. Malaria parasite was maintained by blood passage in NMRI mice when active parasites were required; otherwise it was stored at -70 ºC in Alserver's solution (2.33 % glucose, 0.525 % NaCl and 1 % sodium citrate in deionised water) and glycerol (9:1 parts by volume).

Inoculation of malaria parasites: Mice were inoculated (0.2 ml, i.v.) into a tail vein with blood from a donor mouse (41 % parasitaemia *P. berghei*) diluted with 0.85 % saline to contain 2×10^7 parasitized red blood cells (PRBC). Study on toxicity of herbal extracts on naive mice: Three different concentrations of artediffusin (tehranolide) including 17, 1.7 and 0.17 mg/mL as test animals and a control group (vehicle) all were injected s.c. every day for 8 days with 100 mL solutions. Four groups of mice (n = 5) were investigated for assessment of pathology including body weight, physiological activities, hepatomegaly and splenomegaly.

Antimalarial effects of artediffusin on murine malaria: Antimalarial effects of artediffusin in 70 mg/mL concentration were injected s.c. every day for 12 days after infection in malaria mice. Three groups of mice (n = 5) were investigated for antimalarial efficacy, degree of parasitaemia, assessment of pathology including body weight, physiological activities, hepatomegaly and splenomegaly. Parasitaemia was measured every other day by counting Geimsa-stained blood smears were taken from end tail cutting.

Parasitaemia: The clinical diagnosis was confirmed by laboratory demonstration of the malaria parasite in the stained smears. In all animals parasitaemia was determined on different days after infection using blood smears stained with Geimsa stain (Sigma Chemical Co., USA). Parasitized red blood cells (PRBC) were counted in five different fields, each of approximately 200 cells. Results are expressed as the mean percentage (%) of erythrocytes containing Geimsa positive bodies. Experiments were licensed under the Animals (Scientific Procedures) Act 1986. In compliance with the conditions of this license, infected animals were humanely killed at the onset of the terminal phase of malaria (*P. berghei* NY) infection.

Assessment of degree of hepato/splenomegaly: Entire livers and spleens were removed post mortem at the end of the experimental period from mice after induction of terminal general anaesthesia by inhalation of diethyl ether (Sigma Co., Germany). Organ wet weights were measured and compared with controls as indices for degree of hepatomegaly and splenomegaly.

Measurement of survival rate: Survival rate was presented as the percentage of surviving experimental mice at every other week after inoculation; the significance of differences was determined by statistical test and compared with concurrent appropriate control groups.

Body weight: Body weight was measured initially and at different times of experiment using a top pan balance (OHAUS Scale Corp., USA).

Statistical analysis: Values are presented as the mean ± SEM for groups of n mice. The significance of differences were determined by Student's *t*-test using Graph Pad Prism Software (Graph Pad, San Diego, California, USA) ($p < 0.05$, $p < 0.01$, $p < 0.001$).

RESULTS AND DISCUSSION

Sesquiterpene lactones are found in *A. diffusa* with the peroxide functional group that probably has the same effect of artemisinin as an antimalarial agent. The artemisinin, as a sesquiterpene lactone endoproxide, is becoming an important plant-derived compound in the treatment of the resistant malaria. Although there have been considerable scientific advances over the past hundred years, the overall burden of malaria is currently increasing, especially in sub-Saharan Africa. In the absence of a fully protective antimalarial vaccine, the control of malaria relies principally on the use of drugs for treatment or prophylaxis. Much of the increasing burden of malaria is due to the spread of the resistance of the major human malaria pathogen, *P. falciparum*, to most drugs presently available. Consequently, the World Health Organization and health authorities in malaria-endemic countries are recommending new therapies, based on the use of artemisinin derivatives, combined with other drugs, the so called artemisinin combination therapy. Reports of the resistance of natural populations of *P. falciparum* to these drugs have not been forthcoming thus far, but most agree that resistant parasites will occur in the future, based upon previous experience with other antimalarial drugs. Further isolation and purification of artediffusin is under investigation for antimalarial tests especially *in vitro* activity against the multi drug resistant K_1 strain of *Plasmodium falciparum* and clinical trails to develop new antimalarial agents. Alternatively new therapies can be performed based on the use of combination therapy such as artediffusin combination therapy.

Toxicity in naive mice

Body weight variation as a toxicity effect of artediffusin-therapy in naive mice: The effects of artediffusin on body weight (g). Experimental animals treated with different doses of Artediffusin and control group were administrated with drug vehicle. Values were expressed as mean ± SEM (n = 5) and graphs plotted using graph pad prism Fig. 2.

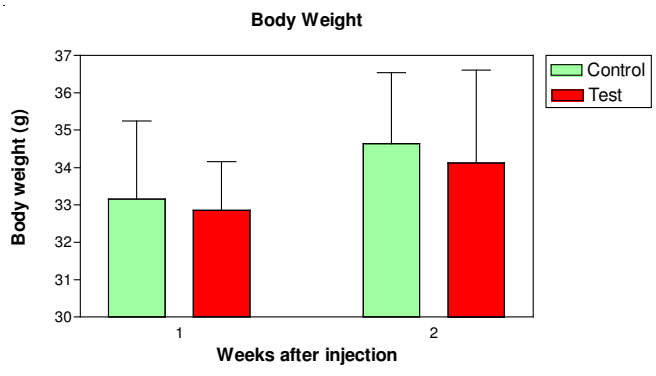


Fig. 2

Hepatomegaly as a toxicity effect of artediffusin-therapy in naive mice: The effects of artediffusin on hepatomegaly (g) was studied by treating experimental animals with different doses of artediffusin and control group were administrated with drug vehicle. Values were expressed as mean ± SEM (n = 5) and graphs plotted using graph pad prism Fig. 2-1.

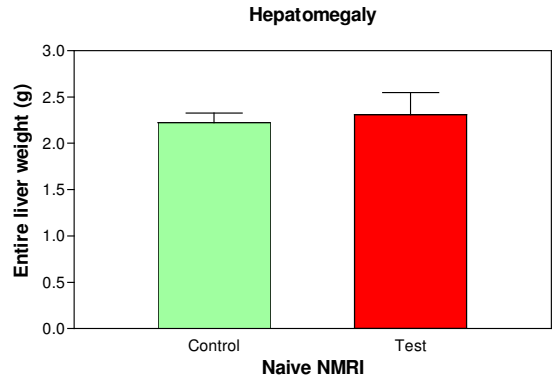


Fig. 2-1

Splenomegaly as a toxicity effect of artediffusin-therapy in naive mice: The effects of artediffusin on splenomegaly (g) was studied by treating experimental animals with different doses of artediffusin and control group were administrated with drug vehicle. Values were expressed as mean ± SEM (n = 5) and graphs plotted using graph pad prism Fig. 2-2.

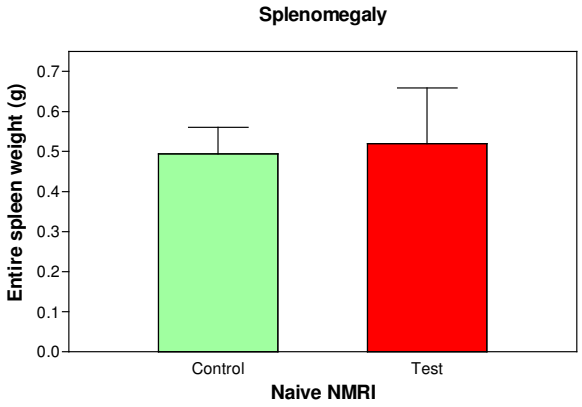


Fig. 2-2

Antimalarial effects in *Plasmodium berghei* infected mice (malaria group)

Antimalarial effects of artediffusin-therapy in malarial mice by measuring parasitaemia percentages: Comparison of parasitaemia percentages during the infection with *P. berghei* in NMRI mice. Mice were inoculated i.v. with 2×10^7 PRBC from a donor mouse infected with *P. berghei* then divided into four groups. Parasitaemia percentages were measured every day by counting Leishman positive cells. Values are presented as means ± SEM (n = 5 mice). Statistical analysis was applied using student's *t*-test with graph pad prism Fig. 3.

Pathophysiological effects in *Plasmodium berghei* infected mice (malaria group)

Body weight variation as a pathological effect of artediffusin-therapy in malarial mice: The effects of Artediffusin on body weight (g) was studied by treating infected NMRI mice with different doses of Artediffusin and control group were administrated with drug vehicle. Values were expressed as mean ± SEM (n = 5) and graphs plotted using graph pad prism Fig. 4.

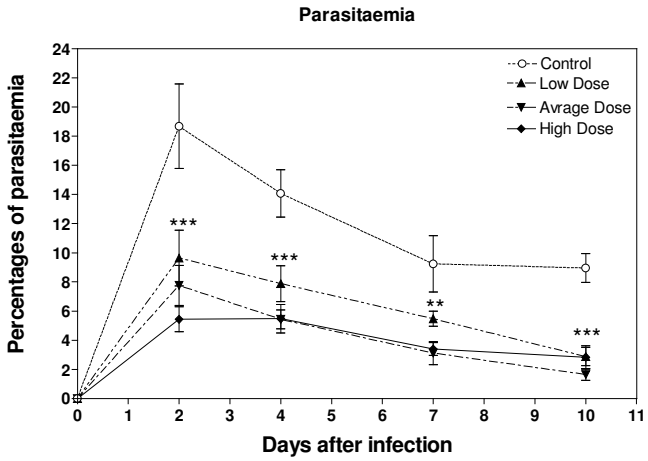


Fig. 3

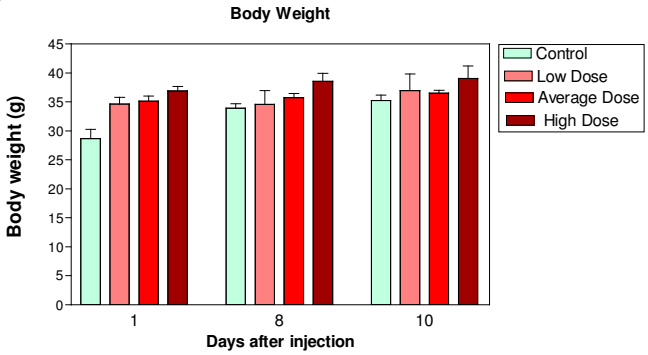


Fig. 4

Hepathomegaly as a pathological effect of artediffusin-therapy in malarial mice: The effects of artediffusin on hepatomegaly (g). Experimental malaria infected NMRI mice treated with different doses of Artediffusin and control group were administrated with drug vehicle. Values were expressed as mean ± SEM (n = 5) and graphs plotted using graph pad prism Fig. 4-1.

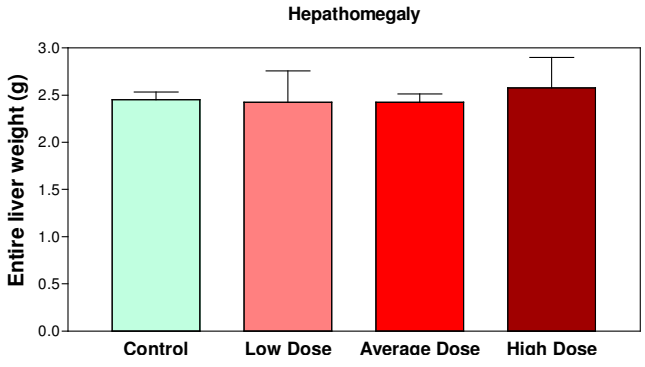


Fig. 4-1

Splenomegaly as a pathological effect of artediffusin-therapy in malarial mice: The effects of artediffusin on splenomegaly (g). Experimental malaria infected NMRI mice treated with different doses of artediffusin and control group were administrated with drug vehicle. Values were expressed as mean ± SEM (n = 5) and graphs plotted using graph pad prism Fig. 4-2.

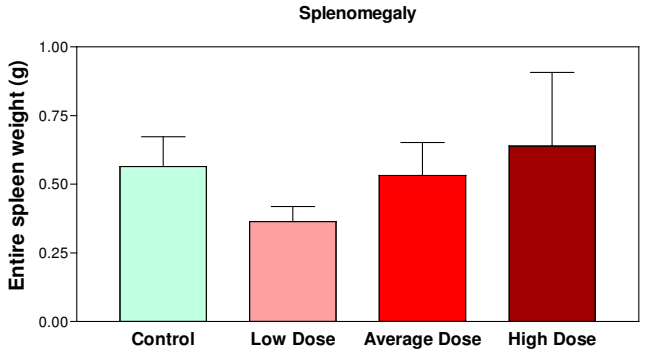


Fig. 4-2

ACKNOWLEDGEMENTS

The authors are grateful to Dr. V. Mozaffarian (Research Institute of Forest and Rangelands) for his assistance in collecting and identifying plant materials.

REFERENCES

1. WHO: World Malaria Report. Available at http:// malaria.wh.Int/wmr2008/
2. S.R. Meshnick, T.E. Taylor and S. Kamchonwongpaisan, *Microbiol. Rev.*, **60**, 301 (1996).
3. B.G. Schuster and W.K. Milhous, *Parasitol. Today*, **9**, 167 (1993).
4. J.F. Trape, A. Pison Spiegel, C. Enel and C. Rogier, *Trends Parsitol.*, **18**, 224 (2002).
5. T.T. Hien and N. White, *Lancet*, **341**, 603 (1993).
6. D.L. Klayman, *Science*, **228**, 1049 (1985).
7. A. Rustaiyan, A. Bamoniri, M. Raffatrad, J. Jakupovic and F. Bohlman, *Phytochemistry*, **26**, 2307 (1987).
8. P. Weyerstahl, S. Schneider, H. Marschall and A. Rustaiyan, *Flavour Fragr. J.*, **8**, 139 (1993).
9. P. Weyerstahl, S. Schneider, H. Marschall and A. Rustaiyan, *Liebigs Annal. Chem.*, 111 (1993).
10. J.A. Marco, J.F. Sanz-Cervera, F. Sancenon, J. Jakupovic, A. Rustaiyan and F. Mohamadi, *Phytochemistry*, **34**, 1061 (1993).
11. A. Rustaiyan, H. Sigari, J. Jakupovic and M. Grenz, *Phytochemistry*, **28**, 2723 (1989).
12. A. Rustaiyan, K. Zare, M.T. Ganji and H.A. Sadri, *Phytochemistry*, **28**, 1535 (1989).
13. J.F. Sanz, A. Rustaiyan and J.A. Marco, *Phytochemistry*, **29**, 2919 (1990).
14. J.A. Marco, J.F. Sanz-Cervera, E. Manglano, F. Sancenon, A. Rustaiyan and M. Kardar, *Phytochemistry*, **34**, 1561 (1993).
15. J.A. Marco, J.F. Sanz, F. Sancenon, A. Rustaiyan and M. Saberi, *Phytochemistry*, **32**, 460 (1993).
16. A. Rustaiyan, S. Balalaei, F. Mohammadi, S. Masoudi and M. Yari, *J. Essential Oil Res.*, **12**, 330 (2000).
17. A. Rustaiyan, H. Komeilizadeh, S. Masoudi, A. Monfared, M. Yari, M. Kardar and M. Shahgholi, *J. Sci. Islamic Republic Iran*, **11**, 213 (2000).
18. F. Sefidkon, A. Jalili and T. Mirhaji, *Flavour Fragr. J.*, **17**, 150 (2000).
19. K. Morteza-Semnani, M. Akbarzadeh and K. Moshiri, *Flavour Fragr. J.*, **20**, 330 (2005).
20. F. Nematollahi, A. Rustaiyan, K. Larijani, M. Nadimi and S. Masoudi, *J. Essential Oil Res.*, **18**, 339 (2006).
21. A. Rustaiyan, H. Nahrevanian and M. Kazemi, Effect of Extracts of *Artemisia diffusa* Against *Plasmodium berghei* as a New Antimalarial Agent, BIT's 5th Anniversary Congress of International Drug Discovery Science and Technology (IDDBST), May 28-June 5, Shanghai, China (2007).
22. A. Rustaiyan, H. Nahrevanian and M. Kazemi, 57th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research, August 16-20, Geneva, Switzerland (2009).