Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(9); 1555-1559

ISSN: 0975-4873

Research Article

Hypolipidemic Effect of *Achillea biebersteinii* Ethanolic Extract in Hamsters with Diet-Induced Hypercholesterolemia

Babelly Mais[1*], Abajy Mohammad Yaser[2], Nayal Ream[3]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Aleppo, Syria.*
[2]*Department of Biochemistry and Microbiology, Faculty of Pharmacy, University of Aleppo, Syria.*
[3]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, University of Aleppo, Syria.*

Available Online: 10th September, 2016

ABSTRACT
Hyperlipidemia is the greatest risk factor of coronary heart disease. The present study was designed to investigate the antihyperlipidemic activity of *Achillea biebersteinii* Afan in high fat diet induced hyperlipidemic Golden-Syrian hamsters. *Achillea biebersteinii* Afan ethanolic extract was administered by oral gavage at a dose of 400mg/kg daily for 20 days to hyperlipidemic hamsters. Atorvastatin was used as reference standard at a dose of 10 mg/kg. *Achillea biebersteinii* Afan showed a significant decrease in the levels of serum cholesterol, triglycerides and LDL. There was also significant decrease in hepatic total cholesterol and triglycerides. According to the results of our study the extract effectively suppressed the high fat diet induced hyperlipidemia in hamsters; this study provides the first report about *Achillea biebersteinii* Afan hypolipidemic effect suggesting the potential protective role in Coronary heart disease.

Keywords: High fat diet, *Achillea biebersteinii* Afan, Hyperlipidemia, Liver, Hamsters

INTRODUCTION

Cardiovascular disorders are one of the major causes of mortality and disabilities worldwide, particularly in developing countries[1]. The distribution of disease is associated with some of its major risk factors as like as hypertension, hyperlipidemia, family history of coronary heart disease CHD, age (> 45 for men, > 55 for women), and smoking. Among these factors, hyperlipidemia has the main role in occurring of CHD, thus control of hyperlipidemia plays a principal part in the protection of CHD[2]. Hyperlipidemia is characterized by elevated serum total cholesterol TC, low density lipoprotein LDL, very low density lipoprotein VLDL and decreased high density lipoprotein HDL levels[3]. Currently available drugs for hyperlipidemia treatment have been associated with many side effects such as myopathy. In that regard, the tendency to use medicinal plants has been doubled[1]. *Achillea* (Yarrow) is one of the most important genus of the Asteraceae family consisting of about 140 perennial herbs native to the Northern hemisphere[4]. The name of the Genus might originate from the name of 'Achilles' from the Greek Mythology since he used this plant to treat his wounds during the Trojan War[5]. Traditional indications of their use include digestive problems, loss of appetite, liver and gall-bladder conditions, menstrual irregularities, cramps, fever and wound healing[6]. *Achillea biebersteinii* Afan, commonly known as "Qaysoum" in Arabic, is one of the predominant *Achillea* species in the Mediterranean region[7]. It is a perennial, herbaceous and aromatic plant, 20 to 50 cm long, usually growing in patches. Heads are yellow, less than 1 cm in diameter, aggregated in a dense flat topped inflorescence[5]. Pharmacological studies have shown that *A. biebersteinii* has different chemical and therapeutical values. In Turkey *A. biebersteinii* has been tested for antifungal and herbicidal properties[8], antimicrobial and antioxidant activity[9,10], wound healing[5], insecticidal[11], protective activity against lipid peroxidation, protein oxidation and DNA damage[12], angiogenesis activity[13]. In Iran this plant has been studied for its anticancerous, antioxidant, and antimicrobial activity[14]. In Jordan the plant was screened for its antioxidant, antibacterial and antiplatelet efficacy[7]. In Egypt protective and therapeutic effect on gastric ulcer and α-amylase inhibitory activity has been evaluated[15]. The purpose of this work was to evaluate the hypolipidemic effect of *Achillea biebersteinii* Afan ethanolic extract by using high fat diet induced hyperlipidemic hamsters.

MATERIALS AND METHODS

Drugs & Chemicals

Cholesterol powder was purchased from Alfa Aesar company, United States. Atorvastatin tablets (10mg) were from Ibn al Haytham Company for pharmaceutical industries, Syria. Cholesterol and triglycerides enzymatically assay kits were purchased from Biosystems Company, Spain. HDL and LDL precipitation assay kits were purchased from MEDICHEM-ME Company.

Plant Material Collection

Aerial parts of *Achillea beibrestinii* Afan were collected while flowering from different regions in Edleb- Syria and identified by Department of Phamacognosy, Aleppo University.

**Author for Correspondence: maisbabelly@gmail.com*

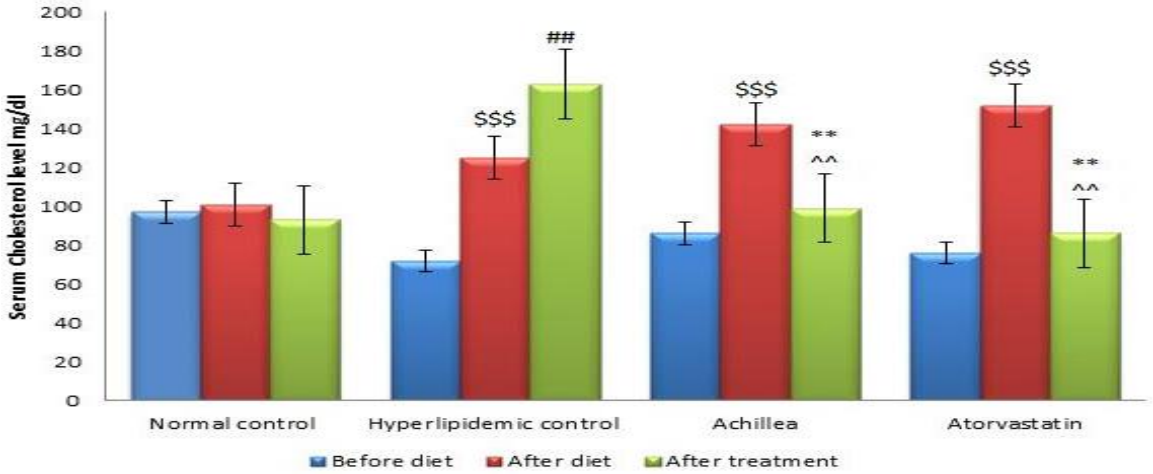

Figure 1: The effect of *Achillea biebersteinii* Afan ethanolic extract on serum total cholesterol levels. Values are expressed as mean ±SD of five hamsters per group. $$$ p<0.001 vs. before diet cholesterol level, ** p< 0.01 vs. Hyperlipidemic control hamsters; ## p < 0.01 vs. Normal control hamsters, ^^ p< 0.01 vs. after diet cholesterol level.

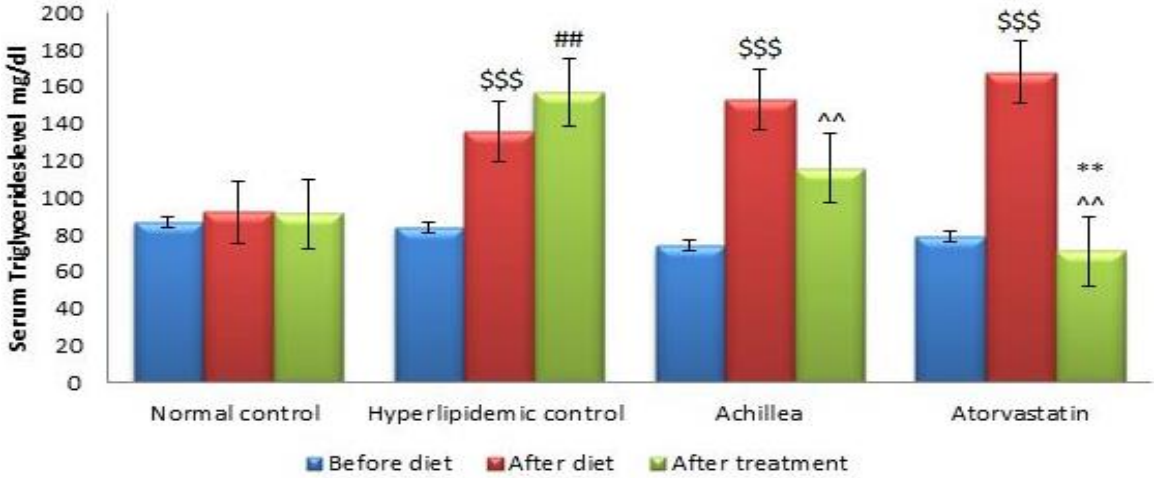

Figure 2: The effect of *Achillea biebersteinii* Afan ethanolic extract on serum triglycerides levels. Values are expressed as mean ±SD of five hamsters per group. $$$ p<0.001 vs. before diet cholesterol level, ** p< 0.01 vs. Hyperlipidemic control hamsters; ## p < 0.01 vs. Normal control hamsters, ^^ p< 0.01 vs. after diet cholesterol level.

The samples were dried at room temperature under a shaded area, ground using an electrical mill, and stored in dark, dry, and cool place.

Extraction Procedure

10 g of plant powder was extracted twice with 200 ml Ethanol: Water (70:30, v/v) using Ultrasound-assisted extraction (Ultrasonic cleaner set, 40 kHz, 150W, model: WUC.A06H, Wiseclean®, Korea). Extraction temperature was 35˚C and extraction time was 35min[16]. After filtration, the pooled ethanolic extracts were evaporated using rotary evaporator under reduced pressure at 40˚C to obtain dry extract.

Selection of Doses for the Study

Dose of *Achillea biebersteinii* Afan was selected according to the acute toxicity study of *Achillea fragrantissima*[17]. The aqueous, methanolic and ethanolic extracts of *Achillea fragrantissima* were tolerated in rats up to 3 g /kg bwt, resulting in no fatality, or any signs of toxicity or change in behavior over 14 days following its administration by oral gavage.

Experimental Animals

Twenty healthy adult male Golden-Syrian hamsters, with an average weight of 100g were obtained from the animal house of the department of Biology, Aleppo University. The animals were housed separately in wire-meshed cages under standard conditions of temperature (25 ± 2˚ C) and 12h light: 12h dark cycle. They were provided with standard dry pellet diet and water *ad libitum*. The hamsters were acclimatized to laboratory condition for 2 weeks before beginning of the experiment.

Investigating The Antihyperlipidemic Activity

Induction of Hyperlipidemia

Hyperlipidemia was induced in Golden-Syrian hamsters by feeding them high fat diet (2% margarine, 2% sugar, 0.2% cholesterol, wt/wt) for 10 weeks. The animals were divided randomly into four groups of 5 animals with similar average body weights. Hamsters in the control group were fed with a basic diet and other groups of animals with a high-fat/cholesterol diet. After induction of hyperlipidemia (which was confirmed by serum estimation of lipid levels) animals in group 1 (Atorvastatin group) received daily dose of 10mg/kg Atorvastatin (p.o) for 20

Table 1: Effect of *Achillea biebersteinii* Afan ethanolic extract on body weight and liver/body weight ratio of high fat diet induced hyperlipidemia in hamsters.

Group	Body weight before HFD (g)	Body weight after HFD (g)	Body weight after treatment (g)	Liver/body weight ratio
Normal control	109±4	98±15	98.7±13	0.03±0.001
Hyperlipidemic control	127±5.6	130±7	133.7±9	0.04±0.001
Achillea	110±17	118±14	121±11.5	0.04±0.007
Atorvastatin	92±30	108±18	110.3±13.9	0.033±0.003

Values are expressed as mean ±SD of five hamsters per group.

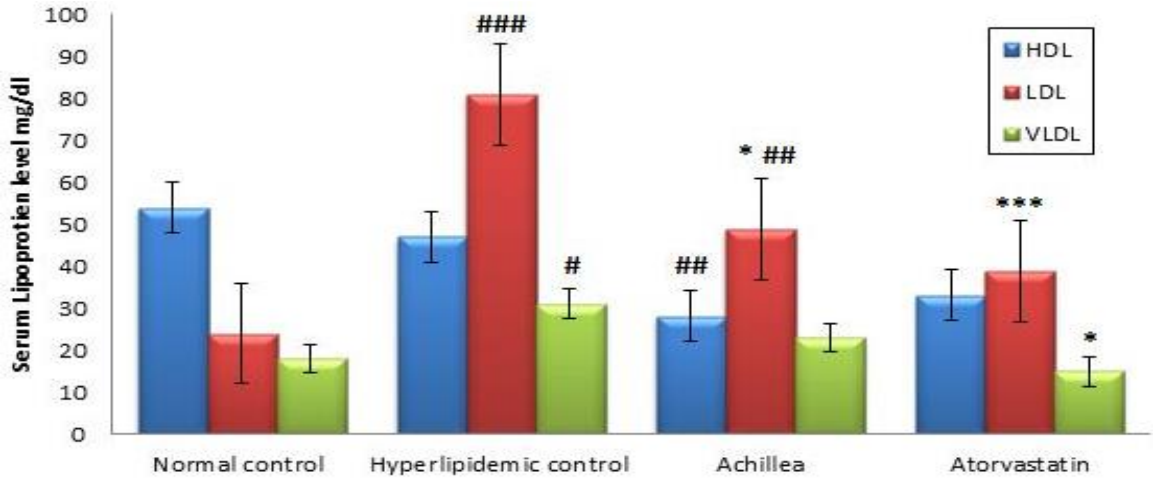

Figure 3: The effect of *Achillea biebersteinii* Afan ethanolic extract on serum HDL, LDL and VLDL levels. Values are expressed as mean ±SD of five hamsters per group. ** $p< 0.01$, *** $p< 0.001$ vs. Hyperlipidemic control hamsters; # $p< 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. Normal control hamsters.

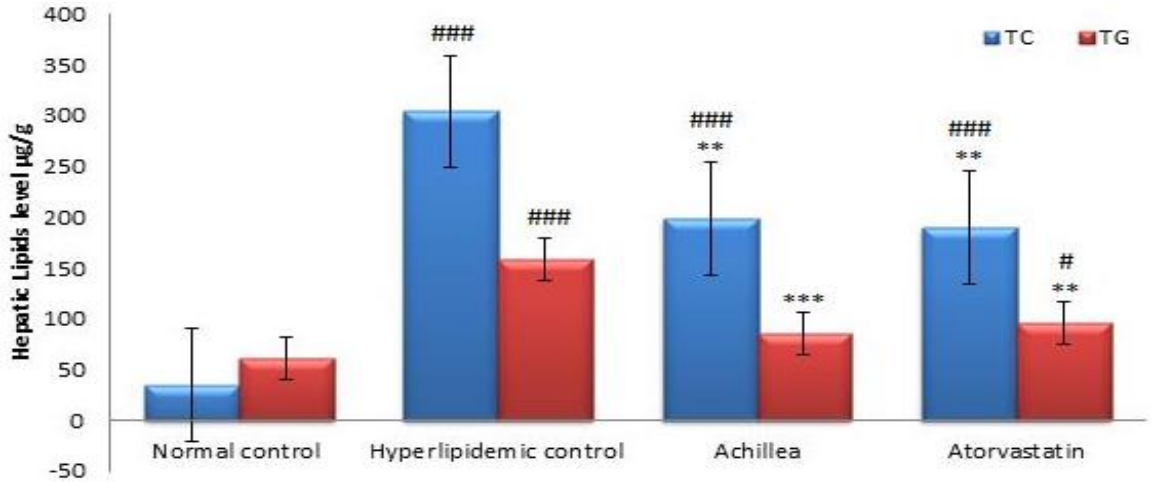

Figure 4: The effect of *Achillea biebersteinii* Afan ethanolic extract on hepatic total cholesterol (TC) and triglycerides (TG) content. Values are expressed as mean ±SD of five hamsters per group. ** $p< 0.01$, *** $p< 0.001$ vs. Hyperlipidemic control hamsters; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. Normal control hamsters.

days. Group 2 (*Achillea* group) was administered daily dose of 400mg/kg *Achillea biebersteinii* Afan dried ethanolic extract suspended in distilled water (p.o) for 20 days, group 3 (Hyperlipidemic group) and group 4 (Control group) received distilled water.

Collection of Blood and Liver Samples

On the 21st day, blood was collected by retero orbital sinus puncture under mild chloroform anesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver was collected, weighed and rinsed with a physiologic saline solution, and immediately stored at -20°C.

Liver Lipid Extraction

The hepatic lipids were extracted using the procedure developed by Folch *et al* (1957). Briefly, 1g of the frozen

tissue was homogenized with a motor driven homogenizer in 20 ml chloroform/methanol (2:1). After homogenization, lipids were further extracted by rocking samples for 15 min at room temperature, followed by filtering. The liquid phase was washed with 0.2 volume of 0.9% saline. Then the lower phase containing lipids was isolated using a separation funnel. After evaporation the dry residue of lipids was dissolved in 0.5 ml isopropanol containing 10% Triton X-100 for triglycerides TG and total cholesterol TC measurements[18]. The cholesterol and Triglycerides concentrations were analyzed with the same enzymatic kits which were used in the serum analysis.

Biochemical Studies

Serum cholesterol, triglycerides, LDL and HDL levels were estimated by enzymatic methods using kits.

VLDL was calculated using the formula [VLDL = Triglycerides/5].

Statistical Analysis

The results were expressed as Mean ± SD. Statistical analysis involving all four groups were carried out by analysis of variance (one-way ANOVA and One-way repeated-measures ANOVA) followed by Turkey's test, *P* value < 0.05 is considered as statistically significant. Data were processed with SPSS software 21st ed.

RESULTS

Changes in Body and Liver Weights

After 10 weeks of high fat diet feeding, hamsters' body weights increased about 3% with no significant differences between groups (Hyperlipidemic, *Achillea*, Atorvastatin, and Control group). Also, at the end of the experiment there was neither significant difference between the 4 groups, nor increase in the liver/body weight ratio as shown in (Table 1).

Serum and Hepatic Lipids

As shown in Figure 1, feeding hamsters with high fat diet for 10 weeks has successfully induced hyperlipidemia by increasing the serum TC and TG levels ($p<0.001$). The treatment with *Achillea biebersteinii* Afan ethanolic extract at a dose of 400mg/kg for 20 days significantly reduced the elevated total cholesterol levels ($p<0.01$) when compared with Hyperlipidemic group and with cholesterol levels for *A. biebersteinii* group after high fat diet period, but there was no significant difference with Normal control or Atorvastatin group, and it reduced the triglyceride levels ($p<0.01$) vs. triglyceride levels for *Achillea* group after high fat diet period (Figure 2). The extract also significantly reduced serum LDL levels ($p<0.01$), although it has no effect on HDL, the extract reduced VLDL levels, but without significant difference (Figure 3). The change in lipid levels in group of Atorvastatin treated hamsters was comparable with Hyperlipidemic and Normal control groups, while there was no significant difference with *Achillea* group levels (Figure 1, 2 and 3). On the other hand, the oral dose of *A. biebersteinii* extract led to significant reductions in hepatic total cholesterol and triglyceride levels ($p<0.01$, $p<0.001$, respectively) compared with Hyperlipidemic group (Figure 4).

DISCUSSION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases[19]. Though there are a large class of hypolipidemic drugs used in the treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effects. Hence efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Amongst the natural resources, medicinal plants hold promise in the discovery of new drugs[20]. *Achillea biebersteinii* Afan is a medicinal plant largely used in the traditional medicine in our area and has been studied widely for its therapeutic effects but there were no studies on the effect of *Achillea* on lipid metabolism and hyperlipidemia. In the present study, we demonstrated that *Achillea biebersteinii* Afan ethanolic extract at a dose of 400mg/kg markedly reduced the serum levels of total cholesterol, triglycerides, LDL and VLDL levels, without any effect on HDL levels, in addition hepatic total cholesterol and triglycerides have been decreased comparing with the Hyperlipidemic hamsters group. The hypolipidemic effect of *Achillea biebersteinii* Afan ethanolic extract may be due to the constitutes of the plant such as sesquiterpene lactones, polyphenols, flavonoids and the essential oil that contain piperitone (31.06%), camphor (12.46%), and eucalyptol (10.98%)[9]. Flavonoids and phenolic compounds were reported to possess antioxidant and other important pharmacological activities including antihyperlipidemic and antihypertensive activity[1]. Numbers of studies in rats have suggested that these compounds may also influence atherogenesis through an effect on lipid and lipoprotein metabolism, and cause significant reduction in serum total cholesterol, triglycerides LDL and VLDL21. In this study *A. biebersteinii* didn't affect HDL levels. This may be due to the short period of extract administration. ASGARY S et al reported that giving *Achillea Wilhelmsii* to 120 volunteers (men and women, aged 40-60 years) significantly increased HDL levels after 6 months' treatment[22]. The reduction in hepatic cholesterol and triglycerides is considered a result of serum LDL levels reduction, which leads to reduction in hepatic synthesis of the cholesterol and triglycerides.

CONCLUSION

Oral administration of *Achillea biebersteinii* Afan ethanolic dry extract at a dose of 400mg/kg to Hyperlipidemic hamsters for 20 days decreased the elevated levels of serum total cholesterol, triglycerides and LDL caused by the hypercholesterolemic diet without any significant effect on HDL cholesterol. In addition, it caused reduction in hepatic total cholesterol and triglycerides. The results of the present study provide the evidence for the antihyperlipidemic activity *Achillea biebersteinii* Afan ethanolic extract. Further studies on the exact mechanism of action, isolation of the active constituents and clinical studies are needed.

ACKNOWLEDGEMENTS

The authors are thankful to the research laboratory, Department of Phamacognosy, Faculty of Pharmacy - Aleppo University, and to Aleppo University Hospital for providing the technical and financial support of the research.

REFERENCES

1. Rouhi-Boroujeni *et al*. Herbs with anti-lipid effects and their interactions with statins as a chemical Anti-hyperlipidemia group drugs: A systematic review. ARYA Atheroscler 2015; Volume 11, Issue 4.
2. Hasani-Ranjbar *et al*. The Efficacy and Safety of Herbal Medicines Used in the Treatment of Hyperlipidemia; A Systematic Review. Current Pharmaceutical Design, 2010, Vol. 16, No. 26.
3. Chacko N *et al*. Anti hyperlipidemic activity of *Costus Igneus* in Triton X- 100 Induced Hyperlipidemic Rats. International journal of pharmaceutical and chemical sciences, Apr – Jun 2012, Vol. 1.
4. Nemeth and Bernath. Biological Activities of Yarrow Species (*Achillea* spp.). Current Pharmaceutical Design, 2008, Vol. 14.
5. Esra K¨upeli Akkol *et al*. Evaluation of the Wound Healing Potential of *Achillea biebersteinii* Afan. (Asteraceae) by In Vivo Excision and Incision Models. Evidence-Based Complementary and Alternative Medicine 2011.
6. Ghani A,Azizi M *et al*. Essential Oil Composition of *Achillea Eriophora*, A. *Nobilis*, A. *Biebersteinii* and A. Wilhelmsii from Iran. Jeobp 11 (5) 2008 pp 460 – 467.
7. Hammad M *et al*. Biological activities of the hydro-alchoholic and Aqueous extracts of *Achillea biebersteinii* Afan. (Asteraceae) grown in Jordan. Afr. J. Pharm. Pharmacol. 8 July, 2013, Vol. 7(25), pp. 1686-1694.
8. Kordali S *et al*. Antifungal and herbicidal properties of essential oils and N-hexane extracts of *Achillea gypsicola Hub-Mor*. And *Achillea biebersteinii* Afan. (Asteraceae). Industrial crops and products 2 9 (2 0 0 9) 562–570.
9. BARIŞ Ö *et al*. Biological Activities of the Essential Oil and Methanol Extract of *Achillea biebersteinii* Afan. (Asteraceae). Turk J Biol 30 (2006) 65-73.
10. Yildirim B *et al*. Volatile Oil Components and Antibacterial Activity of *Achillea biebersteinii* Afan. From Lake Van Basin, Turkey. Asian Journal of Chemistry; 2015, Vol. 27, No. 12, 4686-4688.
11. Çakır A *et al*. Phytotoxic and Insecticidal Properties of Essential Oils and Extracts of Four *Achillea* Species. Rec. Nat. Prod. (2016) 10:2 154-167.
12. Kizil M, Kizil G,Yavuz M and Çeken B. Protective activity of ethanol extract of three *achillea* species against lipid peroxidation, Protein oxidation and dna damage in vitro. Acta Alimentaria, 2010, Vol. 39 (4), pp. 457.
13. Demirci F, Kiyan HT, Demirci B, Başer KHC. The in vivo angiogenic evaluation of *Achillea biebersteinii* Afan. And *Achillea teretifolia Willd*. Essential oils. Planta Med 2011; 77 - PL75.
14. Salar Bashi D *et al*. Investigation of optimal extraction, antioxidant, and Antimicrobial activities of *Achillea biebersteinii* and *A. Wilhelmsii*. Pharmaceutical Biology, 2012; 50(9): 1168–1176.
15. Abd-Alla H I *et al*. Phytochemical composition, protective and therapeutic effect on gastric ulcer and a-amylase inhibitory activity of *Achillea biebersteinii* Afan. Arch. Pharm. Res, 2015.
16. Bashi *et al*. Optimization of Ultrasound-assisted Extraction of Phenolic Compounds from Yarrow (*Achillea beibrestinii*) by Response Surface Methodology. Food Sci. Biotechnol. 2012, VOL:21(4):1005-1011.
17. Mandour M *et al*. The acute and long-term safety evaluation of aqueous, Methanolic and ethanolic extracts of *Achillea Fragrantissima* Afr. J. Pharm. Pharmacol, August, 2013, Vol. 7(32) pp. 2282-2290, 29.
18. Lian Z *et al*. A novel AMPK activator, WS070117, improves lipid metabolism discords in hamsters and hepg2 Cells. Lipids in Health and Disease 2011, 10:67.
19. Yokozawa T *et al*. The Protective Role of Chinese Prescription Kangen-karyu Extract on Diet-Induced Hypercholesterolemia in Rats. Biol. Pharm. Bull. 29(4) 760—765 (2006) Vol. 29, No. 4.
20. Sumalatha G, Vimochana B, Usha rani Ch. Evaluation of antihyperlipidemic activity of ethanolic extract of stem bark of glycosmis Pentaphylla. IJPRD, 2014; Vol 6(04): June-2014 (036 - 044).
21. Mukherjee PK. Plant products with hypocholesterolemic potentials. Advances in Food and Nutrition Research 2003; 47.
22. Asgary S *et al*. Antihypertensive and antihyperlipidemic effects of *achillea wilhelmsii*. DRUGSEXPTL.CLiN. RES. XXVI (3) 89-93,2000