

**EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF ETHANOL LEAF
EXTRACT OF *ORIGANUM MAJORANA* AND *VITEX NEGUNDO* ON
STREPTOZOTOCIN INDUCED DIABETIC RATS**

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ABSTRACT

Medicinal plants are considered as rich resources of ingredients which can be used in new drug development in pharmacopoeial, non-pharmacopoeial or synthetic drug. To compare anti-hyperglycaemic effect between two ethanolic extracts of selected plants locally available in Bobbili region, Vizianagaram district, Andhra Pradesh, India on Streptozotocin induced diabetic rats. To compare anti-hyperglycaemic study, experimental rats were divided into six groups viz. Group I (Normal control), Group II (Disease control, Streptozotocin induced, 50mg/kg b.wt, i.p.), Group III (Diabetic with Glibenclamide, 4mg/kg), Group IV (Diabetic with *Origanum majorana* ethanol extract) and Group V (Diabetic with *Vitex negundo* ethanol extract). Both plants extracts were administered with same dose i.e. 100mg/kg b.wt, orally. The blood glucose levels, lipid profile, body weight were evaluated in all above experimental groups before and after diabetes induction in 21 days study. Significantly decreased blood glucose level and simultaneously improved lipid profile and body weight in Group IV rats after oral administration of ethanol extract whereas in Group V rats are showing less effect compare to Group IV rats. Both the plant extracts effect compare with Group II and Group III rats statistically. *Origanum majorana* shows better antihyperglycaemic activity because the leaf contain huge amount of phytoconstituents like rutin and quercetin isolated flavonoids. In *Vitex negundo* leaves ethanol extract also shows antihypoglycaemic activity because it contains 1,2- disubstituted idopyranose, β -sitosterol like active constituents. *Origanum majorana* and *Vitex negundo* plants may be used as a dietary supplement in diabetic patients. Further study is required to evaluate the antihyperglycaemic activity.

KEYWORDS: *Origanum majorana*, *Vitex negundo*, Hyperglycaemia, Phytoconstituents.

INTRODUCTION

Plants have been used for therapeutic purposes long before prehistoric period. Therapy with medicinal plants is considered very safe as there is no or minimal adverse effects. The herbal products are the symbol of safety in contrast to the synthetic drugs.^[1,2] In this study two medicinal plants are selected for their antihyperglycaemic activity on streptozotocin induced diabetic rat model.

Origanum majorana (Family-*Lamiaceae*) is an aromatic herb in the mint family.^[3,4] It has many medicinal uses with huge health benefits. It improving digestion,

preventing intestinal infections, relieving diarrhoea and constipation^[5,6], also used in a variety of common illnesses such as food poisoning, staph infection, tetanus infection in wounds, typhoid, malaria, influenza, common cold, mumps, etc.^[7] From ethnopharmacological study it was reported different pharmacological activities like antiseptic, antidote^[8], antioxidants^[9,10,11], anticonvulsant^[12,13], anti-anxiety^[14], antimicrobial, antibacterial, antifungal^[15,16], antiulcer^[17], antiprotozoal, insecticidal, antiovicidal^[18], anti-gout^[19], antidiabetic^[20], antimutagenic, antitumor^[21,22], anti-inflammatory, analgesic.^[23] etc.

Vitex negundo Linn (Family- *Verbenaceae*) is a large aromatic shrub distributed throughout India which is extensively used as traditional medicine, folk medicine and pharmacological evidence.^[24] The most important pharmacological screening activities are anti-inflammatory^[25], anti-rheumatic^[26], antibiotic, hepatoprotective^[27], antioxidant, anticonvulsant, oxidative stress^[28,29] snake venom neutralization and anti-allergic activities.^[30,31]

On qualitative test ethanol leaves extracts contain numbers of secondary metabolites such as alkaloids, phenols, flavonoids, glycoside, tannin, terpenes, linalool, cacacrol, etc. which may responsible for different medicinal activities.

Origanum majorana and *Vitex negundo* shows many medicinal effects which are useful for treatment of different common disease. Quercetin, rutin, 1,2-disubstituted idopyranose, β -sitosterol like isolated compounds are present in ethanolic leaves extracts that may shows antidiabetic and hypolipidemic activities.^[32,33]

MATERIALS AND METHODS

Collection and Identification of Plants

The fresh *Origanum majorana* and *Vitex negundo* leaves were collected in the month of October 2015 from the

forest of Bobbili region, Vizianagaram district, Andhra Pradesh, India, authenticated by Dr. Madhava Chetty, Department of Botany, S.V. University, Chittor Dist. Tirupati. The leaves were deposited in the Herbarium of Department of Botany.

Preparation of Plant Extracts

Plant materials were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried then made into fine powder. These powdered samples (100g/500ml) in ethanol for 48 hours at 45°C. The Phytochemical constituents are extracted by using Soxhlet apparatus. The extracts were soaked and evaporated under pressure and concentrated at 50°C and the residue obtained was stored at 4°C.

Preliminary Phytochemical Screening

Specific qualitative tests were performed to identify bioactive compounds such as tannins, alkaloids, saponins, flavonoids, terpenoids and phenols were determined from ethanol extracts of *Origanum majorana* and *Vitex negundo*.

Table 1: Phytochemical Screening of ethanol extracts of selected plants.

SL No.	Phytochemical constituents	Ethanol extract of <i>Origanum majorana</i>	Ethanol extract of <i>Vitex negundo</i>
1	Alkaloids	+++	+++
2	Flavonoids	+++	+++
3	Tannins	++	+
4	Saponins	+++	+++
5	Glycosides	++	+
6	Steroids	+	++
7	Resins	+	-
8	Phenols	+++	++
9	Cardiac glycosides	++	+++
10	Terpenoids	++	+

Experimental animals

The animals used in experiment were procured from animal house of Nalla Narasimha Reddy Education Society's Group of Institution, from Pharmacy Department. Wistar rats of either sex weighing about 160–200 g were taken. Experimental protocols were approved by the Institutional Animal Ethic Committee (CPCSEA NO.-282/P0/Bt/S/2000). The animals were kept in polycarbonate cages and maintained under standard housing conditions of temperature (22 ± 20C) and humidity (45–60%) with 12 h light-dark cycle. Animals were fed pellet diet with supply of water *ad libitum* and normal saline. Animals were divided into five different groups as normal control, disease control, reference group, and test groups.

Acute Oral Toxicity Study

Acute oral toxicity studies of ethanol extracts of *Origanum majorana* and *Vitex negundo* were carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD) no. 423. As per OECD guidelines minimum number of animals should be used (3 animals per dose) for experiment to obtain the information as acute toxicity of test dose.^[34] Overnight fasted rats were orally fed with plant extract at a dose level of 250, 500, 1000 and 2000 mg/kg body weight respectively. The animals were observed continuously for 2hrs to investigate any sign of toxicity, occasionally for 4 hrs for their general behavior and after a period of 24 hr, animals were observed for any sign of mortality till 7 days.^[35]

Induction of diabetes experimentally

Wistar rats (160-200gm) were fasted for 18 hours before the induction of diabetes with Streptozotocin (STZ), for induction of Type-1 Diabetes mellitus. Animals (n=30) were injected intraperitoneal with 0.22-0.25ml of freshly prepared solution of STZ (50 mg/ml in 0.01 M citrate buffer, pH 4.5) at a final dose of 50 mg/kg body wt. The diabetic state was assessed in STZ-treated rats by measuring the non-fasting serum glucose concentration after 72 hours. Only rats with serum glucose levels greater than 200-250 mg/dl were selected and used in this experiment.

Experimental Design for Oral Glucose Tolerance Test (OGTT)

In oral glucose tolerance test, animals of diabetic control group have shown significant elevation in blood glucose level through entire study when compare to normal animals. But treatment with standard drug glibenclamide and ethanol extracts (100 mg/kg) of *Origanum majorana* and *Vitex negundo* could able to reduce significantly ($P < 0.01$) blood glucose level in therapeutic groups after 60 mins and 120 mins.^[36] The results of OGTT have shown in [Table No 2].

Streptozotocin-induced Diabetic Model

The animals were divided into five groups of six rats each. The ethanolic extracts were administered for 21 days. Group I served as normal control rats administered sodium carboxy methyl cellulose (SCMC) daily for 21 days; Group II diabetic control rats administered STZ (50mg/kg) with SCMC, Group III diabetic rats administered standard drug glibenclamide (4 mg/kg); Group IV diabetic rats administered *Origanum majorana* (100 mg/kg); Group V diabetic rats administered *Vitex negundo* (100 mg/kg). The fasting glucose levels were determined on days 1, 7, 14 and 21 of extracts administration. During the experimental period, the blood glucose level, the lipid level and body weight of different group animals are estimated.^[36,37]

Estimation of Biochemical Parameters

The biochemical parameters were determined on day 21 after the animals were sacrificed by cervical dislocation. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) were determined by the glucose oxidase method, using an auto-analyzer.^[38]

Statistical Analysis

Results of estimation of biochemical and functional parameters have been reported as mean value \pm SEM. The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet's test (Sigma stat 3.5). P values < 0.05 , were considered statistically significant.

RESULT AND DISCUSSION

Preliminary Phytochemical Screening: Specific qualitative tests identified the phytochemicals constituents such as tannins, alkaloids, saponins, flavonoids, terpenoids and phenols from ethanol extracts of *Origanum majorana* and *Vitex negundo*.

Acute Oral Toxicity Study

The result of acute toxicity study of ethanol extracts of above plants on laboratory animals showed that no lethality up to the dose of 2000 mg/kg body weight hence the animals were safe up to a maximum dose of 2000 mg/kg body weight.

Oral Glucose Tolerance Test (OGTT)

The effects of ethanolic extracts of selected plants on the plasma glucose level are illustrated in table 2. Both ethanolic extracts showed significant reduction in plasma glucose level in rats at 90 minutes and same was observed in standard drug at 60mins. 100 mg/kg body weight of both plants extracts treated rats produces significant reduction in plasma glucose level, while in disease control rats, plasma glucose level was increased.

Effect of Ethanolic Extract on Streptozotocin-induced Diabetic Rats

The diabetes rats were confirmed with increasing level of fasting plasma glucose level. The effect of ethanol extracts at same dose (100mg/kg) of *Origanum majorana* and *Vitex negundo*, on fasting plasma glucose level of normal and streptozotocin induced are given in table 2. The difference between the experimental and control rats in lowering the fasting plasma glucose levels were statistically significant by compare with diabetic rats.

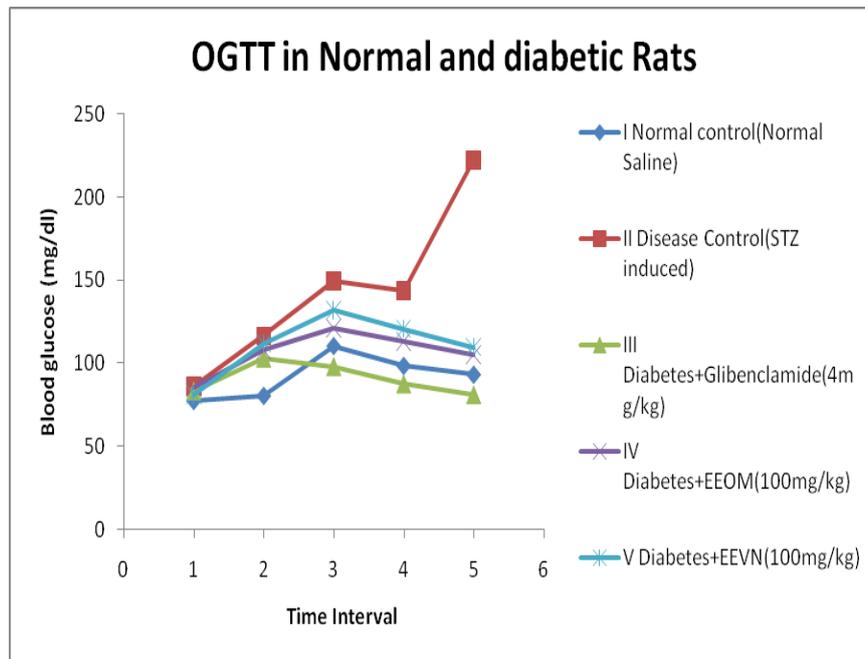
Effect of Ethanolic Extract on Biochemical Parameters in Streptozotocin-induced Diabetic Rats

Both plants ethanolic extracts on diabetes induced hyperlipidemia were also evaluated. It was observed that due to diabetes there was an increase in the total cholesterol levels as well as triglyceride levels. The HDL levels were reduced in the diabetic animals and the LDL levels were increased significantly (Table 4). The ethanol extracts showed a significant decrease in the total cholesterol levels and triglyceride levels. It also increased the HDL level and was successful in suppressing the LDL and VLDL levels as compared to the standard drug (Table 4). Compared to both plant extracts *Origanum majorana* shows better effect compare to *Vitex negundo*.

Table 2: Effect of ethanolic extracts of *Origanum majorana* (EEOM) and *Vitex negundo* (EEVN) (100 mg/kg, PO), on oral glucose tolerance test (OGTT) in normal and streptozotocin induced diabetic rats.

Groups	Treatment of Dose(mg/kg)	BLOOD GLUCOSE LEVEL (mg/dl)				
		Fasting	30 mins.	60 mins.	90 mins.	120 mins.
I	Normal control(Normal Saline)	77.5±0.63	80.5±0.59	110.39±0.66	98.5±0.71	93.25±0.51
II	Disease Control(STZ induced)	86±0.88	116.45±0.84	149.5±0.72	143.8±0.68	222.5±0.47
III	Diabetes+Glibenclamide(4mg/kg)	83.5±0.82	103.05±1.02	98.08±1.52	87.62±1.05	81.25±0.98
IV	Diabetes+EEOM(100mg/kg)	85.6±0.71	107.9±0.69	121.05±1.01	113.25±0.75	105.24±0.73
V	Diabetes+EEVN(100mg/kg)	81.25±0.59	112.24±0.81	132.4±1.36	121.5±0.93	110.25±0.89

Values are given as Mean ± SEM for n=6. Group IV was compared with group I and II. Group IV and V were compared with group II. Values are statistically significant at **p< 0.01. EEOM-Ethanol extract of *Origanum majorana*, EEVN-Ethanol extract of *Vitex negundo*.

**Figure-1: Effect of *Origanum majorana* and *Vitex negundo* ethanol extracts on OGTT in normal and diabetic rats.****Table-3: Effect of multiple dose treatment of ethanolic leaf extract of *Origanum majorana* and *Vitex negundo* (100 mg/kg, PO), (Once daily), on blood glucose level after 21 days in normal and Streptozotocin induced diabetic rats.**

GROUPS	Treatment of Dose (mg/kg)	BLOOD GLUCOSE LEVELS (mg/dl)				
		Basal value	Day 1	Day 7	Day 14	Day 21
I	Normal control(Normal Saline)	78.23±0.73	87±0.52	98±0.28	91±0.54	85±0.69
II	Disease Control(STZ induced)	248.57 ±1.85	252.5±2.15	259.8±1.89	263.5±2.24	266.25±2.71
III	Diabetes+Glibenclamide(4mg/kg)	240.36±2.29	228.52±2.14*	154.2±0.16**	130.05±1.29**	101±1.412**
IV	Diabetes+EEOM(100mg/kg)	244.37 ±2.98	235.15±2.85*	162.8±2.45**	140.25±1.24**	125.5±0.54 **
V	Diabetes+EEVN (100mg/kg)	245.05±2.57	239.35±2.71	178.26±2.42**	156.15±1.75**	131.65±1.62*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05, when compared with diabetic control group.

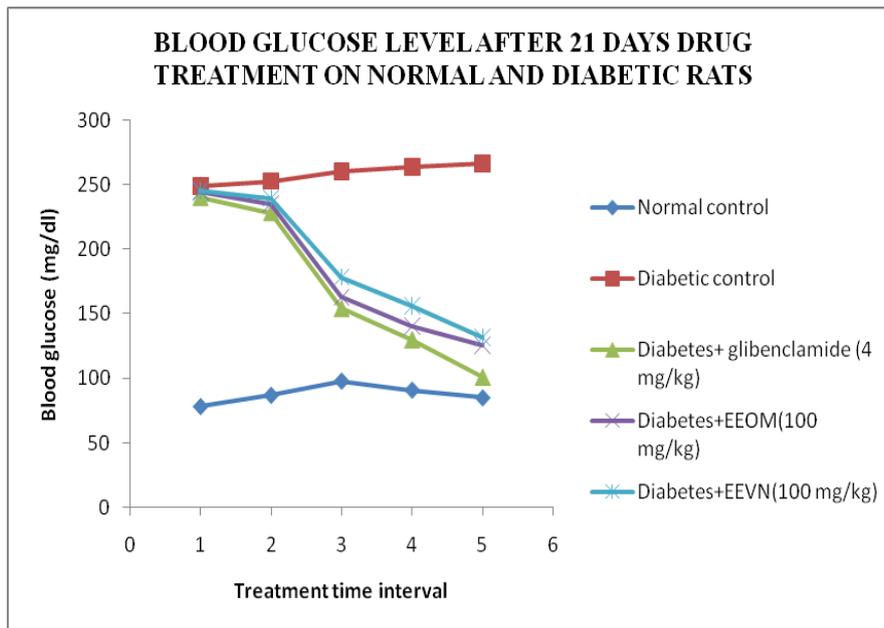


Figure-2: Effect of *Origanum majorana* and *Vitex negundo* ethanol extracts on blood glucose level in diabetic rats.

Table 4: Effect of ethanolic leaf extract of *Origanum majorana* and *Vitex negundo* (100 mg/kg, PO) on Serum Biochemical Parameters after 21 days treatment.

GROUPS	TREATMENTS	LIPID PROFILES (mg/dl)				
		TC	HDL	LDL	VLDL	TG
I	Normal Control	136±1.89	43.25±0.75	92±0.81	16.23±0.62	72.84±0.96
II	Disease control (STZ induced)	184.6±0.91	20.17±0.39	109.7±1.62	18.89±0.56	98±2.86
III	Diabetic+ glibenclamide (4mg/kg)	151.05±1.49***	44±1.25***	82.55±2.92***	17.65±0.37***	84.2±1.55***
IV	Diabetic +EEOM (100mg/kg)	169.23±1.24	37.18±1.39**	96.40±1.65*	21.05±0.35**	89.21±2.56*
V	Diabetic +EEVN (100mg/kg)	171.25±1.52	34.17±1.53	101.43±1.58	23.12±0.21	94.47±2.35

The values indicate mean ±S.E.M (n=6). p<0.05 compared with normal control values and p<0.05 compared with diabetic control values and test drugs.

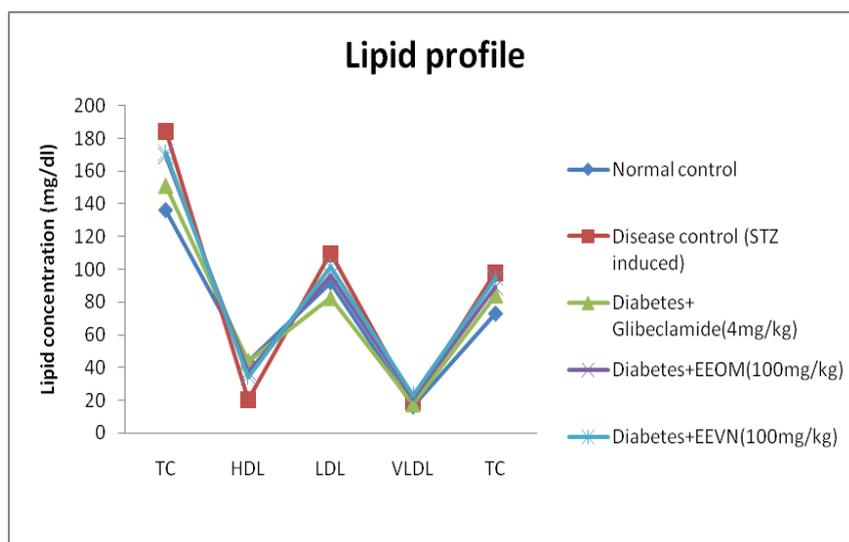


Figure-3: Effect of *Origanum majorana* and *Vitex negundo* ethanol extracts on lipid profile and compared with normal and diabetic rats.

DISCUSSION

From the above study it was observed that OGTT results (Figure. 1), that leaves ethanol extract of *Origanum majorana* reduced greater glucose concentration, where

as *Vitex negundo* shows less response comparatively. Ethanolic leaves extract *Origanum majorana* gives significant decrease in the blood glucose level after 7th day treatment but *Vitex negundo* shows less response

during OGTT, but after prolonged administration of extract it significant decrease in the blood glucose level also which indicating anti-diabetic potential but percentage reduction in the blood glucose level is less than *Origanum majorana* leaves extract (Figure 3). It was investigated that the secondary metabolites such as flavonoids, saponins and triterpenes are beneficial to control blood glucose level and these phytoconstituents are present in both plant extracts confirmed by qualitative test. The consumption of flavanoids or flavanoid rich foods reduces the risk of diabetes mellitus. It was reported that Rutin, quercetin and eridictyol like isolated flavonoid components are present in leaves extract of *Origanum majorana* which shows positive antidiabetic and hypolipidemic activities.^[39,40] In case of *Vitex negundo*, it is also shows antidiabetic activity because of 1,2- disubstituted idopyranose, β -sitosterol like phytoconstituents are present which maintain blood glucose level in diabetic condition.^[22] In the above study *Origanum majorana* ethanol leaves extract comparatively shows better response then leaves ethanol extract of *Vitex negundo*. On the other hand, Saponins also present in both the plants and it have the ability to reduce increased plasma blood glucose level hence; it may useful in treatment of diabetes mellitus.^[32,33] Similarly, the presence of terpenoids inhibit enzymes involved in glucose metabolism and prevent the development of insulin resistance thus normalising insulin levels.^[41] A marked increase in serum concentration of TC, LDL, VLDL, TG and decreased HDL was observed with diabetic rats than normal control group which is generally known as hyperlipidaemia.^[42] From this study it was reveals that the administration of EEOM and EEVN not only lowered TC, LDL, VLDL and TG but also enhanced the cardioprotective lipid HDL.

CONCLUSION

From above study it was revealed that *Origanum majorana* leaves has significant anti-hyperglycaemic potential and the ethanol leaves extract of this plant may be used as herbal medicine which is substitute for synthetic drugs to treat diabetic patients. On the other hand prolonged treatment with *Vitex negundo* ethanol leaves extract also showed reduction in the blood glucose level and maintain lipid profile, it can may also be used as a anti-hyperglycaemic drug with lesser potential than *Origanum majorana*.

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REFERENCES

1. www.nofa.org/tnf/summer 2012B.pdf.
2. <http://www.who.in/medicines/areas/traditional/select mono vol4.pdf>
3. Vagi E, Simandi S, Daood H, Deak A, Sawinsky J. Recovery of pigment from *Origanum majorana* L. by extraction with supercritical carbon dioxide. J Agri Food Chem, 2002; 50: 2297-2301.
4. *Origanum majorana* and *Origanum vulgare* spp, Hirtum growing in India, Chem Nat Comp, 2012, 47,6 Shirley P, The aromatherapy workbook, Hammersmith.
5. Bruneton J, Pharmacognosy, Phytochemistry, Medicinal Plants, Technique and Documentation, Lavoisier, Paris, 1999; 3: 1120.jh.
6. Dudus L E, Bodrug M V, Nemtsanu N N and Kokosh E N, Shashlik sauce, USSR Patent No. 906497, 1982.
7. Kordali S, Cakir A, Ozer H, Cakmaki R, Kesdek M and Mete E, Antifungal phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum occidentis* and its three components, carvacrol, cymol, p-cymene, Bioresour Technol, 2008; 99(18): 8788-8795.
8. Cano J H and Volpato G, Herbal mixtures in the traditional medicine of Eastern Cuba, J Ethnopharmacol, 2004; 90: 293-316.
9. Ryszard A, Zofia Z, Ryszard R, Ronald B P, Magdalena K and Agnieszka K, Antioxidant activity and free radical-scavenging capacity of ethanol extracts of thyme, oregano and marjoram, Eur J Lipid Sci Tech, 2009; 111: 1111-1117.
10. Gulcin I, Antioxidant and antiradical activities of Lcarnitine. Life Sci., 2006; 78: 803-811.
11. Vagi E, Rapvi E, Hadolin M, Vasarhelyine Perdei K, Balazs A, Blazovics A and Simandi B, Phenolic and triterpenoid antioxidants from *Origanum majorana* L., herb and extracts obtained with different solvents, J Agric Food Chem, 2005; 53: 17-21.
12. Senevirathne M, Kim SH, Siriwardhana N, Ha JH, Lee KW and Jeon YJ, Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. Food Sci Technol Int., 2006; 12: 27-38.
13. Deshmane D N, Gadgoli C H and Halade G V, Anticonvulsant effect of *Origanum majorana* L., Pharmacologyonline, 2007; 1: 64-78.
14. Hosseinzadeh H, Naassiri AM. Anticonvulsant, sedative and muscle relaxant effects of carbenoxolone in mice. BMC Pharmacol, 2003; 3: 3-13.
15. Wilson, J., W.P Watson and H.J. Little, CCK (B) antagonist protect against anxiety related behavior produced by ethanol withdrawal, measured using the elevated plus maze. Psychopharmacology, 1998; 137: 120-131.
16. Mohamed N, Yasmen S H and Nohir G, Antimicrobial activity of water and ethanol Marjoram (*Origanum marjorana* L.) extract, The 6th

- Arab and 3rd International Annual Scientific Conference, 2011; 2350- 2366.
17. Sharma N, Dubey N K and Sharma K, Screening of insecticidal and antifungal activity of *Origanum majorana* oil against *Callosobruchus chinensis* L. and *Aspergillus* spp., *Res J Agric & Biol Sci.*, 2011; 7(2): 223-227.
 18. Kozłowska M, Laudy A E, Starosciak B J, Napiorkowski A, Chomicz L and Kazimierzuk Z, Antimicrobial and antiprotozoal effect of sweet marjoram (*Origanum majorana* L.), *Acta Scientiarum Polonorum-Hortorum Cultus*, 2010; 9(4): 133-14.
 19. Vasudeva N, Singla P, Das S and Sharma S K, Antioxidant and antioxidant activity of stem and root of *Origanum majorana* Linn, *Am J Drug Discov Dev*, 2014; 4(2): 102-112.
 20. Martha R, Gutierrez P, Inhibition of advanced glycation end-product formation by *Origanum majorana*, in vitro and in streptozotocin-induced diabetic rats, *J Evid Based Complement Altern Med*, 2012; 1: 1-8.
 21. Baricevic, D., Milevoj, L. and Borstnik, J. Insecticidal effect of oregano (*Origanum vulgare* L. ssp. *Hirtum* Ietswaart) on the dry bean weevil (*Acanthoscelides obtectus* Say). *Int. J. Horticultural Sci.*, 2001; 7(2): 84–88.
 22. Hashim, E.F., Seham, K.A.A. and Kheir, A.A. Nematicidal activity of some labiateous plant extracts on *Meloidogyne incognita*. *Ann. Agric. Sci. Cairo*, 1999; 44(1): 447–457.
 23. Van Den Broucke, C.O. and Lemli, J.A. Antispasmodic activity of *Origanum compactum*. *Planta Medica*, 1980; 38: 317–331.
 24. (2014) *Vitex negundo* Linn. Fact Sheet. Bureau of Plant Industry, Department of Agriculture, Republic of the Philippines. <http://www.stuartxchange.com/Lagundi.html>
 25. Telang, R.S., Chatterjee, S. and Varshneya, C. (1999) Studies on Analgesic and Anti-Inflammatory Activities of *Vitex negundo* Linn. *Indian Journal of Pharmaceutical Sciences*, 31: 363-366.
 26. Kirtikar, K.R. and Basu, B.D. (1984) *Indian Medicinal Plants*. Bishen Singh Mahendra Pal Singh, Dehradun.
 27. Yang, L., Yen, K., Kiso, Y. and Hikino, H. (1987) Antihepatotoxic Actions of Formosan Plant Drugs. *Journal of Ethnopharmacology*, 103-110.
 28. Tiwari, O.P. and Tripathi, Y.B. (2007) Antioxidant Properties of Different Fractions of *Vitex negundo* Linn. *Food Chemistry*, 1170-1176.
 29. Gupta, M., Mazumder, U.K. and Bhawal, S.R. (1999) CNS Activity of *Vitex negundo* Linn. in Mice. *Indian Journal of Experimental Biology*, 37: 143-146.
 30. Samy, R.P., Thwin, M.M., Gopalakrishnakone, P. and Ignacimuthu, S. (2008) Ethnobotanical Survey of Folk Plants for the Treatment of Snakebites in Southern Part of Tamilnadu, India. *Journal of Ethnopharmacology*, 115: 302-312.
 31. Zabihullah, Q., Rashid, A. and Akhtar, N. (2006) Ethnobotanical Survey of Kot Manzary Baba Valley, Malakand Agency, Pakistan. *Pakistan Journal of Plant Sciences*, 12: 115-121.
 32. Yang, J. Y., Della-Fera, M. A., Rayalam, S., Ambati, S., Hartzell, D. L., Park, H. J., and Baile, C. A. (2008) Enhanced inhibition of adipogenesis and induction of apoptosis in 3T3-L1 adipocytes with combinations of resveratrol and quercetin. *Life Sci.*, 82: 1032–1039.
 33. Chandramu, C., Rao, D.M., Krupanandam, D.G.L. and Reddy, D.V. (2003) Isolation, Characterization and Biological Activity of Betulinic Acid and Ursolic Acid from *Vitex negundo* L. *Phytotherapy Research*, 17: 129-134.
 34. Rosen JS, Cleary JE. Surgical management of wounds. *Clin. Podiatr. Med. Surg*, 1991; 8: 891-907.
 35. OECD, 2000. Acute Oral Toxicity-Acute Oral Toxic Class Method. Guideline 423, adopted In: Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, 1996.
 36. Vogel G, Vogel H. The text book of pharmacological screening methods and drug evaluation. Evaluation of anti-diabetics. Springer link publishers, 947-60.
 37. B. Singh, N. Dutt, D. Kumar, S. Singh, R. Mahajan, *J. Adv. Dev. Res*, 2011; 2(1): 21-29.
 38. C. S. Umesh, K. Yadav, K. Moorthy, Z. B. Najma, Combined treatment of sodium orthovanadate and *Momordica charantia* fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats. *Mol cell Biochem*, 2005; 268: 111-120.
 39. A. K. Maurya, S. Tripathi, M. Kahrana, Z. Ahmed, and R. K. Sahu, "Evaluation of antidiabetic effects of *Ficus racemosa* extracts in diabetic induced rats," *Pharmacologyonline*, 2011; 3: 907–914.
 40. Baatour O, Kaddour R, Mahmoudi H, Tarchouni, Bettaieb I, Nasri N, Mrah S, Hamdaoui G, Lachaal M and Marzouk B, Salt effects on *Origanum majorana* fatty acid and essential oil composition, *J Sci Food Agric*, 2011; 10: 1002-4495.
 41. Olalekan O, Saponins E. Anti-diabetic principles from medicinal plants- A review. *J Pathophysiol*, 2015; 22(2): 95-103.
 42. C. S. Umesh, K. Yadav, K. Moorthy, Z. B. Najma, Combined treatment of sodium orthovanadate and *Momordica charantia* fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats. *Mol cell Biochem*, 2005; 268: 111-120.