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BIOLOGICAL EVALUATION OF CRUDE PLANT EXTRACTS FOR ANTIADIPOGENENIC ACTIVITY IN 3T3 L1 CELLS

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Abstract
Obesity is an increasing health problem globally. Phytocostituents are potential agents to inhibit differentiation of preadipocytes, stimulate lipolysis and induce apoptosis of existing adipocytes. In this *in-vitro* study, *Croton bonplandianum* (Ban Tulsi), *Origanum majorana* (Sweet marjoram), *Vitex negundo* **(**Nirgundi) and *Indigofera linnaeia*, (Birdsville Indigo) ethanolic extracts of the selected plants were screened for their cytotoxicity and antiadipogenic activities. For testing the *in-vitro* cytotoxicity of the extracts, an MTT assay was performed with concentrations of test extracts ranging from 10 to100 µg/mL. Effect of the plant extracts on lipid accumulation in cultured 3T3-L1 adipocytes was determined by measuring Oil Red O staining. Among the four plant extracts, *O.majorana* and *I.linnaei* ethanolic extracts showed higher cytotoxicity activity and significantly reduced the lipid droplets accumulation with 72.81% and 59.23% over control at test concentration100µg/mL respectively through the quantification method of Oil Red O staining. These two plant extracts were also effective to reduce triglyceride accumulation with significant values 58.51% and 54.62% respectively. Other two extracts namely *Croton bonplandianum* (19.26%) and *Vitex negundo* (11.27%) did not show significant inhibition of lipid and triglyceride accumulation at the tested concentration compare with standard drug Simvastatin (78.24% and 71.42%). By HPLC analysis, the present study revealed that *O.majorana* and *I.linnaei* were possessed quercetin and kaempferol like important phytoconstituents which may exhibits antiadipogenic activity by reducing lipid and triglyceride accumulation. Consumption of standardized herbal plant extracts may be safe and effective treatment for obesity and related metabolic disorder diabetes.

Keywords: Antiadipogenic, Cytotoxicity, MTT assay, Simvastatin, Plant extracts,

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INTRODUCTION

Many indigenous plant extracts have been found to have different biochemical properties which reported to possess anti-adipogenic activity with beneficial effects on health, and these plant extracts have drawn concentrate because of their relative cost effectiveness, safety as compared to synthetic drugs and their pharmacological properties such as anti-obesity and anti-diabetic effects [1,2]. Reduced adipocyte differentiation is one of the important mechanisms for anti-adipogenesis [3]. Quercetin and Kaempferol in different plant extracts have been reported to suppress adipogenesis in 3T3-L1 adipocytes and different types of rutin containing extracts suppresses differentiation of 3T3-L1 preadipocytes and obesity in high fat diet fed rats [4, 5, 6]. Adipocyte differentiation in over-weight individuals with increased fat mass can affect cell size produce adipose tissue hyperplasia which results adipogenesis [7, 8]. Preadipocyte cell lines are useful models for investigating the adipogenesis process. 3T3-L1 preadipocyte, which can be induced to differentiate into adipocyte cells, is one of the most studied preadipocyte cell line [9, 10]. During adipocyte differentiation, transcriptional factor like PPARγ (peroxisome proliferator-activated receptor-γ), CCAAT element binding protein (C/EBPs) are responsible for the sequential expression of adipocyte-specific proteins. So C/EBP is expressed at the onset of preadipocyte differentiation [11, 12]. PPARγ and C/EBP these two components are produce expression of adipocyte-specific proteins, are induced adipocyte differentiation [13, 14, 15]. The cytotoxicity assay depends on the reduction of tetrazolium salt, MTT (3-(4, 5–d-imethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide), by living cells to form a blue formazan product. This cytotoxicity assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used [16, 17]. In this study, we screened crude extracts of four plants as potential anti-adipogenic agents through an *in-vitro* study. *Croton bonplandianum* Baill., (Family Euphorbiaceae) due to the resemblance of the leaves and flower cymes to that of Tulsi, this plant is often called Ban tulsi locally [18, 19]. It is a medicinal herb used in many health related problems like cholera, boils, bowel complaints, diarrhoea, dysentery, insanity, acute constipation, abdominal dropsy, internal abscesses, cold and cough, lungs infection, bronchitis, asthma, jaundice, liver complaints, reduce pain, sprains,

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headache. It is also used for the treatment of scurvy, malaria, chicken pox, eye diseases, skin diseases, rheumatism, epilepsy and many other diseases [20, 21, 22]. The leaves extract contains secondary metabolites are alkaloids, saponins, steroids, flavonoids, tannins, terpenoids and phenolic compounds [23, 24]. *Origanum majorana* (family-Lamiaceae), commonly known as Sweet marjoram, Majorana hortensis or Marwa in India. Marjoram has many uses with numerous health benefits [25]. Digestive benefits **(**Increasing the efficiency of digestion by increasing digestive enzymes and saliva, improving appetite, relieving nausea, eliminating flatulence, preventing intestinal infections, relieving diarrhea and constipation) [26, 27]. Marjoram is a great antiseptic, antibacterial, antifungal, and antiviral agent and used in a variety of common illnesses (Food poisoning, Staph infection, Tetanous infection in wounds, Typhoid, Malaria, Influenza, Common cold, Mumps, Measles) [28]. Another benefit of marjoram is the enhancement of the cardiovascular and circulatory system (Lowering the blood pressure, greatly reducing the risk of hypertension, preventing the buildup of cholesterol).Anti-inflammatory effects like (Asthma, Muscle spasms, Sinus headaches, Migraines, Fever, Body aches) [29]. Topical application for (Painful joints, Sore muscles, Sprains, Back aches, Toothaches , Emotional and neurological benefits like (Relieving insomnia, Reducing stress, Calming anxiety, Minimizing emotional reactions, Increasing control of sexual desire) [30, 31].The herb contains important phyto constituents like tannins, glycosides, terpenes, flavonoids, linalool and cavacrol [32]. *Vitex negundo* (family- Verbenaceae) commonly known as the five-leaved chaste tree, is a large aromatic shrub, widely used in folk medicine [33]. *In vitro* and animal studies have shown that chemicals isolated from the plant have potential anti-inflammatory [34], antibacterial, antifungal and analgesic activities. *Vitex negundo* is used for treating stored garlic against pests and as a cough remedy in the Philippines [35, 36]. Roots and leaves used in eczema, ringworm and other skin diseases, liver disorders, spleen enlargement, rheumatic pain, gout, abscess, backache; seeds used as vermicide. It is also used to control population of mosquitoe [37]. Traditionally the leaves of *Vitex negundo Linn.* are documented to possess antibacterial, antitumor, astringent, febrifuge, sedative, tonic and vermifuge. It has been reported to posses' potent pharmacological properties like antiinflammatory, anti-rheumatic, antibiotic, Hepatoprotective, antioxidant, anticonvulsant, oxidative stress, anti-androgen, snake venom neutralization and anti-allergic activities. The various chemical constituents like flavonoids, flavones glycosides, volatile oil, triterpenes, tannins and many others were identified in this plant [38, 39].

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The plant *Indigofera linnaei Ali* (family-Fabaceae), commonly known as Birdsville indigo [40] is a Trailing, branched, slender annual or perennial herbs with woody rootstock, 15–50 cm high with a long taproot. Leaves imparipinnate. *Indigofera* is a large genus of over 750 species of flowering plants. They are widely distributed throughout the tropical and subtropical regions of the world. *Indigofera linnaei* is one of important species used to alleviate pain. The herbs are generally used for toothache, insect stings, snake bites and swellings, relieve ulcer pain. anthelmintic, tonic, skin disorders, for toothache, ulcer, solid tumors, epilepsy, anti-nociceptive, analgesic and anti-inflammatory, antioxidant, rheumatism, arthritis, antimicrobial, antidyslipidemic, anti-fertility, antiscorbutic, diuretic, jaundice and to treat burns, liver disease and psychiatric illness, promoting growth of hair, chronic bronchitis, asthma, hydrophobia, in gastropathy and also used as thermogenic, laxative, expectorant etc [43]. The above plants were also found to be used by the tribals in different parts of India for various disorders including obesity. because these plants contains important phytoconstituents like rutin, Quercetin, kaempferol, luteolin, gallic acid, β-Sitosterol like which are may responsible for antiadipogenic activity [44]. Hence we elucidated antiadipogenic effects on lipid and triglyceride accumulation in cultured 3T3-L1 adipocytes by measuring Oil Red O staining as indicators. There are no previous reports of these plant extracts being screened for their lipid inhibitory activity.

MATERIALS AND METHODS

Plant collection and authentication:

Fresh plant materials were collected from forest region of Bobbili, Vizianagaram (District), Andhra Pradesh, India. And they were identified and authenticated by Dr. Madhava Chetty, Department of Botany, S. V. University, Chittoor District, Tirupati and leaves were deposited in the Herbarium of Department of Botany.

Preparation of alcoholic extracts of the selected plants

Croton bonplandianum, Origanum majorana, Vitex negundo* and *Indigofera linnaeia:

Preparation of Plant Extract: Freshly collected plant material was dried under shade and coarsely powdered in a willey mill. The coarsely powdered material (500 g) was extracted with petroleum ether to remove the fatty material and further extracted in a Soxhlet apparatus and subjected to continuous extraction with ethanol (95%). The extract was filtered and concentrated by using rotary flash vaccum evaporator and evaporated under pressure and concentrated at 50°C and the residue obtained was stored at 4°C. The concentrate was thoroughly air dried to remove

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all traces of the solvent and the percentage yield was calculated .The extractive value of the extraction was obtained by using the relation,

% Extraction = Weight of concentrate / Weight of fresh material X 100

Preliminary Phytochemical Screening: Different qualitative phytochemical tests exhibited for the presence of flavonoids, tannins, glycosides, cardiac glycosides, sterols, terpenoids in ethanol extract of above plant materials which are presented in table-1.

The above plant extracts are evaluated and analysed using sophisticated modern techniques of standardization such as TLC, HPLC, GC-MS, LC/MS etc.

***In-vitro* cytotoxicity of the selected plant extracts in 3T3-L1 cells**

For testing the *in-vitro* cytotoxicity of the selected plant extracts, an MTT assay was performed to assess the cell viability with concentrations of test extracts of the selected plants ranging from 10 to 100 µg/mL.

Chemicals:

For cytotoxicity study used reagents are Glucose, Antibiotics, Trypsin, DMEM (Dulbecco's Modified Eagle's Medium), DMSO (Dimethyl Sulfoxide), PBS (Phosphate Buffered Saline), FBS (Fetal Bovine Serum), EDTA (Ethylenediamine tetra acetic acid), MTT (3-(4, 5–d-imethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide), etc.

Cell lines and culture medium:

3T3-Ll cell lines were received from National Centre for Cell Science (NCCS), Pune, India. In a humidified atmosphere of 5% CO_2 at 37°C, the 3T3-L1 cell line was cultured until cell confluent in DMEM culture media which is supplemented with penicillin (100 IU/mL), amphotericin B (5mg/mL), Fetal Bovine Serum (FBS), streptomycin (100mg/mL). By TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) the confluent cells were dissociate. In 25cm^2 culture flasks the stock cultures were emerge and in 96 microtitre plates the entire test carried out.

Preparation of test solutions:

The test samples were must prepare in liquid form for cytotoxicity study. The concentration of 1mg/ml drug made up with DMEM complete media with 2% FBS to prepare a stock solution of 100mg/ml concentration and sterilized by filtration and centrifuges. Serial fivefold dilution was prepared from the stock solution.

Procedure:

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.2 ml of the

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diluted cell suspension (approximately 20,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off and the monolayer was washed once with medium and 100 ml of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^0 C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 48 h, the drug solutions in the wells were discarded and 50 ml of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 ml of isopropanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC50) is generated from the dose-response curves for each cell line.

% Growth Inhibition =

100 – [Mean OD of individual test group / Mean OD of control group] x100

In-vitro anti-adipogenic studies of selected plant extracts in 3T3- L1 cells

The anti-adipogenic studies were carried out on 3T3- L1 cells and the cytotoxicity effect of plant extracts on inhibition of lipid droplet accumulation was determined by quantification of Oil Red O staining method.

Chemicals:

Fetal Bovine serum (FBS), glucose, insulin, dexamethasone, Oil Red O stain and antibiotics.

Equipments:

Automated Microplate reader, CO_2 Incubator, Inverted Tissue culture Microscope (Olympus) were used for the Anti-adipogenic studies.

Cell lines and culture medium:

Until confluent the preadipose 3T3L1 cell lines was cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with penicillin (100 IU/mL), streptomycin (100 mg/mL), amphotericin B (5 mg/mL), 10% inactivated fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 at 37°C. The cells were detached from the tissue culture flask by using Phosphate Buffered Saline (PBS) which consists of 0.2% trypsin, 0.02% EDTA, 0.05% glucose. The stock culture was grown in 25 cm^2 culture flask and all experiments were carried out systematically.

DOI: 10.21276/IJIPSR.2019.07.01.764

Preparation of test solutions:

For adipogenesis studies, the test extracts were weighed and made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 100 mg/mL concentration and sterilized by filtration and centrifuged. Serial fivefold dilution was prepared from the stock solution.

Culture and differentiation: (Steps for lipid droplets accumulation)

Subculture and seedling of preadipocyte cell line for induction of differentiation: 3T3-L1 preadipocyte cells were cultured in DMEM. When the cells are around 70-80% confluent, harvested the cells from the 25 cm^2 tissue culture flask by trypsinization. The cells are seeded in complete media in 96 well plates. After 2 days cells were grown up to 100% confluence, the cells are kept another 48 hours in this state to arrest the cell division. Then adipocyte differentiation media was treated.

Adipocyte differentiation: After post confluency the ceels are feeded with adipocyte differentiation media (complete media with 1µM Dexamethazone, 0.5 mM 3-Isobutyl-1-methylxanthine, 10µg/mLInsulin) and the cells are kept in this condition for 96 hours.

Adipocyte maturation: After 96 hours all the media discarded and added adipocyte maturation media (complete media with 10 µg/mL insulin) at day 4. Every 2 days the media was change and lipid droplets are visible from inside the cells at day 8. Now the cells are ready for assay. After 8 days, cells were maintained with DMEM with 10% FBS along with test samples with different concentration (12.5, 25, 50, 75, 100 µg/mL) for 48 hours.

Adipocyte differentiation confirmation by Oil Red O Staining and optical density (O.D) measurement: 3T3-L1 adipocytes were washed with phosphate-buffered saline (PBS) and fixed with 10% formalin for 30 min. After washing twice with distilled water, cells were stained for at least 1 h at room temperature in freshly diluted Oil Red O containing 0.5% Oil Red O in isopropanol. Finally, the dye retained in the 3T3-L1 cells was eluted with isopropanol and quantified by measuring the optical absorbance at 520 nm.

Assay for Triglyceride accumulation:

3T3-L1 preadipocytes were differentiated into adipocytes by adding isobutylmethylxanthin, dexamethasone and insulin for 8 days. The cells were treated with or without plant extracts (12.5, 25, 50, 75, 100 µg/mL) and Simvastatine used as standard drug in the dose of 10µg/mL during differentiation period. The triglyceride content of the cells was measured enzymatically by using commercially available kits (Bio Vision Inc., Milpitas, CA). The test sample treated 3T3-L1 cells were homogenized in 5% NP-40 assay buffer and the sample was slowly heated to stabilize all the

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triglycerides. The samples were mixed with lipase and triglycerides reaction mixture. The sample absorbance was measured at 570 nm, after 1 hour of incubation.

RESULTS

Preparation of alcoholic extracts of the selected plants

Croton bonplandianum, Origanum majorana, Vitex negundo and *Indigofera linnaeia:* The alcoholic extracts were obtained which possessed characteristic odour with pungent smell. The percentage yield of the extracts obtained was found to be 11.5 (EECB), 13.25 (EEOM), 9.5 (EEVN), 10.34 (EEIL)

Table 1: Qualitative analysis of phytoconstituents of ethanolic extract of following Selected plants

SL.No.	Active phytoconstituents	*Croton bonplandianum*	*Origanum majorana*	*Vitex negundo*	*Indigofera linnaei Ali*
1	Alkaloids	+++	+	++	+
2	Flavonoids	+++	+++	+++	+++
3	Saponins	++	++	++	+
4	Tannins	++	+	+	++
5	Phlobatannins	-	-	-	-
6	Glycosides	+++	+++	++	++
7	Sterols	++	+	+++	++
8	Resins	+	++	+	+
9	Phenols	++	+	++	+
10	Anthraquinones	-	-	+	-
11	Terpinoids	++	-	++	++
12	Cardiac glycosides	++	++	++	++

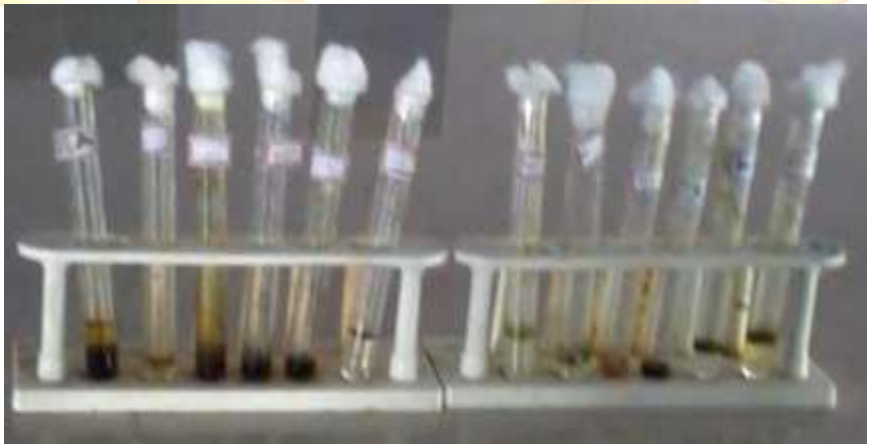

Fig.1: Qualitative phytochemical analysis

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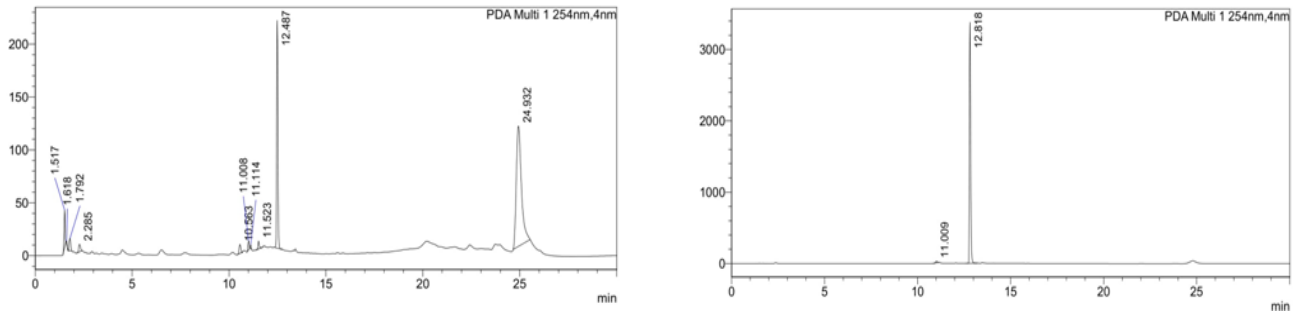
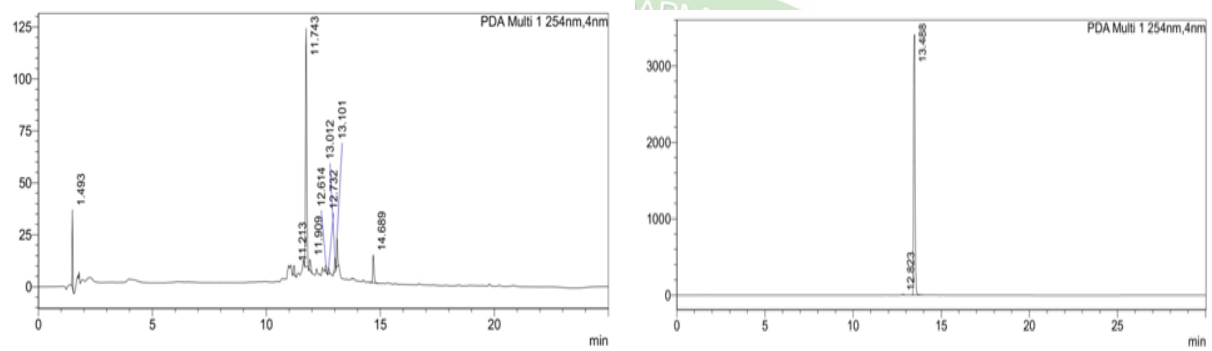


(A) **(B)**

Fig.2: Quercetin from ethanolic extract of *Origanum majorana* (A) compare with marker compound Quercetin (B)



(C) **(D)**

Fig.3: Kaempferol from ethanolic extract of *Indigofera linnaei* (C) compare with marker compound Kaempferol (D)

HPLC Phytochemical analysis

Table 2: *In-vitro* cytotoxicity of the selected plant extracts in 3T3-L1 cells

The results of *In-vitro* cytotoxicity effect of selected plant extracts two plants shows better response compare to other two plants in 3T3-L1 cells were presented in table-2 and Figure-4

Treatments	% Cell viability
Untreated cells	100.00±5.45
DMSO treated	98.84±4.77
Simvastatin(1µM)	46.08±2.01
V1(10µg/mL)	119.68 ±2.36
V1(100µg/mL)	123.15 ±8.00
V2(10µg/mL)	119.68 ±2.36
V2(100µg/mL)	121.83 ±5.48
V3 (10µg/mL)	109.90 ±2.31
V3 (100µg/mL)	106.77±10.85
V4 (10µg/mL)	114.72±5.31
V4(100µg/mL)	119.61±13.91

V1-V2- Ethanolic extract of *Origanum majorana*

V3-V4- Ethanolic extract of *Indigofera linnaei*

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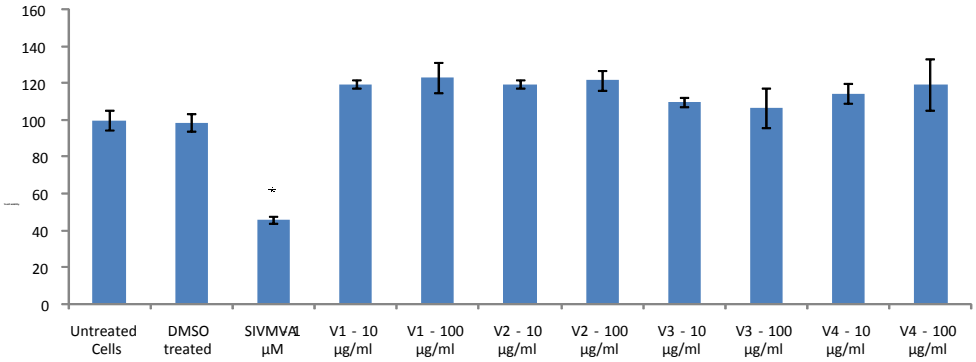

Fig. 4: Cytotoxicity of extracts on 3T3 L1 Cell line

Table 3: Inhibitory effect of selected plants ethanolic extracts on Lipid accumulation by using 3T3L1 adipocytes

Test samples	% inhibition effect of selected plants ethanolic extract on Lipid accumulation				
	Concentrations(µg/mL)				
	12.5	25	50	75	100
EECB	5.38±1.38	7.49±0.97	11.02±1.28	14.53±4.05	19.26±2.45
EEOM	21.05±3.57	38.15±2.84	46.57±1.73	59.45±2.79	72.81±3.25
EEVN	4.61±1.26	6.24±0.59	5.97±2.41	9.05±1.48	11.27±0.96
EEIL	16.64±1.69	31.08±3.27	46.35±4.05	53.14±3.37	59.23±2.78
Standard (Simvastatin)	29.5±2.32	47.21±1.52	56.15±2.57	64.21±1.58	78.24±3.08

EECB- Ethanolic extract of *Croton bonplandianum*, EEOM- Ethanolic extract of *Origanum majorana*, EEVN- Ethanolic extract of *Vitex negundo*, EEIL- Ethanolic extract of *Indigofera linnaei*

Table 4: Inhibitory effect of selected plants ethanolic extracts on Triglyceride accumulation by using 3T3L1 adipocytes

Test samples	% inhibition effect of selected plants ethanolic extract on Triglyceride accumulation				
	Concentrations (µg/mL)				
	12.5	**25**	**50**	**75**	**100**
EECB	6.68±3.72	10.37±5.69	25.19±1.56	28.07±4.28	31.18±0.82
EEOM	16.25±3.34	31.08±2.98	45.34±1.67	52.47±2.56	58.51±3.28
EEVN	7.85±3.83	12.43±5.41	15.37±3.24	9.82±4.18	20.29±3.71
EEIL	22.58±1.88	33.81±3.04	41.21±4.21	48.18±2.57	54.62±3.34
Standard (Simvastatin)	27.34±2.41	42.71±3.15	53.5±2.65	69.82±2.91	71.42±3.12

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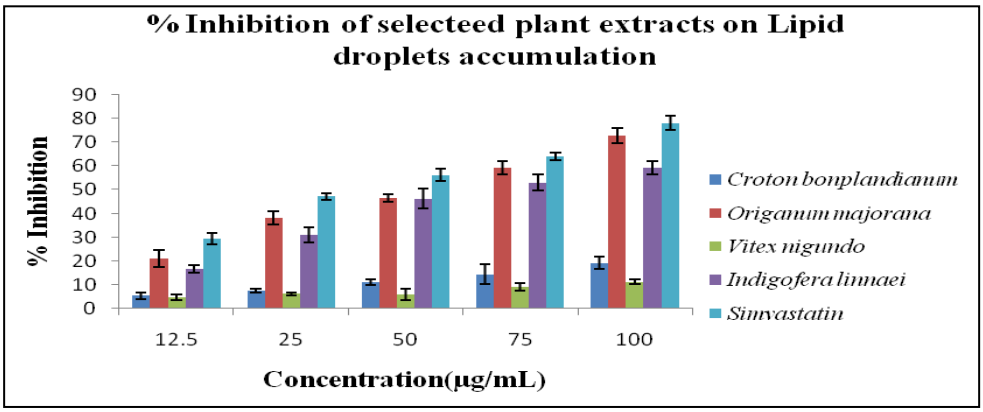

Fig. 5: Inhibitory effect of above selected plant extracts on lipid droplets accumulation related factors in 3T3L1 adipocytes

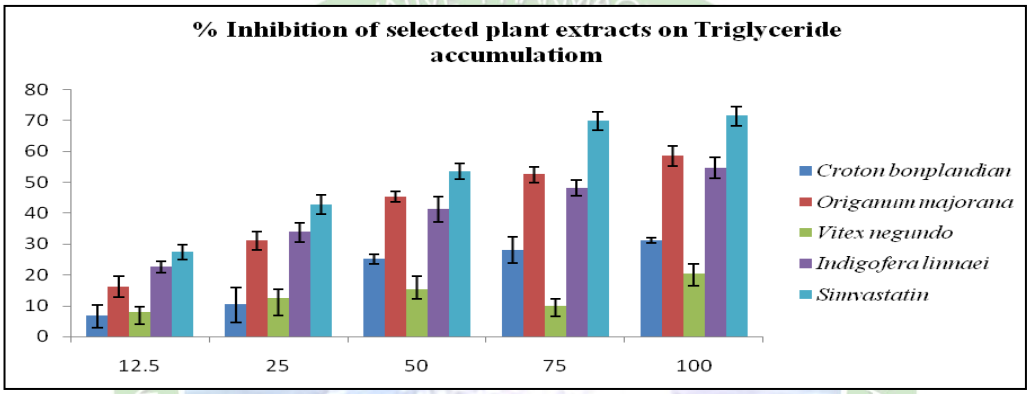

Fig.6: Inhibitory effect of selected plant extracts on Triglyceride accumulation in 3T3L1 adipocytes.

DISCUSSION

Obesity is closely associated with several disorders like coronary heart disease, hypertension, type 2 diabetes mellitus, respiratory complications, and osteoarthritis. The results obtained in the present study by examined on the plant extracts for the first time, the anti-adipogenesis activity of the selected plant extracts in 3T3-L1 preadipocytes without eliciting cytotoxicity of the cells. To examine the effect of plant extracts on cell viability of 3T3-L1 preadipocytes, an MTT assay was performed, which assess cell viability by measuring mitochondrial activity in 3T3-L1 cells, treated with different concentrations of test extracts of the selected plants *Croton bonplandianum, Origanum majorana, Vitex negundo* and *Indigofera linnaeia*. The alcoholic extracts of the selected plants were tested at concentrations of 10 to100 µg/mL. The control sample ethanol showed poor toxicity at tested concentrations of all the extracts test was found to be EEOM and EEIL, there is no toxicity in these two plant extracts but EECB and EEVN plant extracts shows little toxic effect. One of the screening methods used in the discovery of antiadipogenic activity is by measuring Oil Red O staining as indicators of lipid accumulation. The 3T3-L1 adipocytes were

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cultured and differentiated in a Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum for 6 to 8 days in the absence and presence of plant extracts. The effects of plant extracts on lipid droplets formation in 3T3-L1 cells, and inhibition through the quantification of Oil Red O staining. Adipogenesis was substantially inhibited by the test extracts and among the four extracts screened for antiadipogenic activity, test extracts EEOM, EEIL significantly reduced the lipid accumulation with 72.81, 59.23 (%) at test concentration of 100µg/mL respectively through the quantification method of Oil Red O staining. These are significant compared with standard drug Simvastatin (100µg/mL) which inhibit 71.42. Other two extracts namely EECB (19.26%) and EEVN (11.27%) did not show significant inhibition of lipid accumulation at the highest tested concentration. In case of triglyceride accumulation at highest concentration 100µg/mL the tested extracts EEOM, EEIL, EECB and EEVN shows significant reduction of triglyceride accumulation in such manner 58.51, 54.62, 31.18, 20.29 (%). These observations demonstrate that among the four extracts tested for anti-adipogenesis activity *in-vitro*, compared to ethanolic leaf extract of *C.bonplandianum* and *V.nigundo,* the *O.majorana,* and whole plant of *I.linnaei* proved to be beneficial in the management of adipogenesis. By HPLC it was investigated that above two plant extracts contain huge amount of phytoconstituents such as Rutin (quercetin-3-o-rhamnose glycoside), luteolin, luteolin-7-O-glucoside, apigenin, linolenic, linoleic and oleic acid, Kaempferol 3-O-α-L-rhamnopyranoside, Kaempferol 7-O-α-L-rhamnopyranoside, quercitin 7-O-β-D-glucopyranoside, Quercetin 3-O-[β-D-xylo pyrano -syl-(1→2)-β-galactopranoside which are may responsible for antiadipogenesis [45, 46].

CONCLUSION

Due to the worldwide increase in obesity, discovering natural compounds that inhibit the formation of new adipocytes and prevent the accumulation of lipid droplets and triglycerides at various stages of the adipocyte life cycle is a vital provocation of life science. In accordance, from the above *in-vitro* study it was identified that from four plant extract two are produced antiadipogenesis and reduce the accumulation of lipid in differentiated mature adipocytes (present study) and simultaneously reduce triglyceride accumulation. These natural extracts can be used as an easily accessible source of natural antidiabetic with clear effects on adipocyte biology. Universal increase of obesity now challenge for the search alternative treatments for this prevalent. In these circumstances the investigation might have useful in the prevention and/or treatment of obesity and its related complications. In conclusion, from four plants ethanolic

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extracts of *O.majorana* and *I.linnaei* plays a significant role compared to *C.bonplandianum* and *Vitex negundo* in the control of lipid metabolism and adipogenesis, and may helpful for naturally control blood glucose level in diabetic patients.

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