Anti- Adipogenic Properties of Some Selected Plant Extracts on 3T3-L1 (Pre)-Adipocytes.

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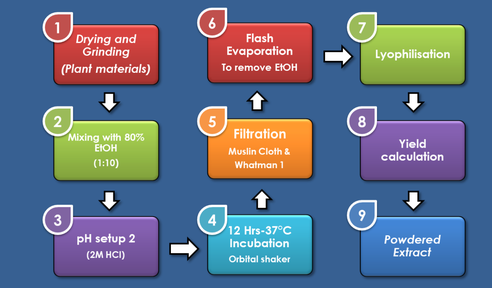
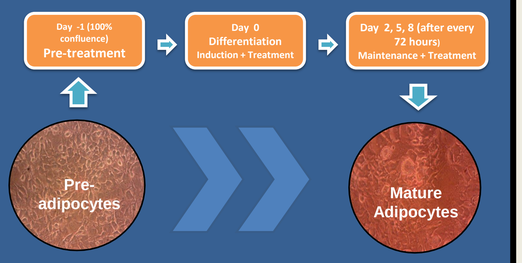
ABSTRACT

Obesity is one of the major health problems worldwide, and it is a risk factor for several chronic disorders. The rising prevalence of obesity has compelled the scientific community to come up with sustainable solutions to address this modern epidemic. Many drugs based treatments have been identified to treat obesity, but having serious side-effects. This necessitates investigating the prospect of natural substances as alternative and complementary means to deal with the present scenario. Numerous studies on bio-active compounds isolated from plant sources have shown promising results against obesity. Present study was undertaken to investigate the effect of crude extracts of some selected plants on 3T3-L1 pre-adipocytes proliferation and their influence on adipogenesis.



***Flow-chart 1*: Extraction procedure for ethanol extract of plants**



***Flow-chart 2*: Pre-adipocyte differentiation**

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OBJECTIVES

- Investigating the effect of some crude plant extracts namely *Moringa oleifera* (MO), *Brassica oleracea* (BO) and *Ocimum basilicum* (OB) on 3T3-L1 pre- adipocyte proliferation and their influence on adipogenesis.
- Incorporating these plant extracts to develop a functional food product – Anti-obesity soup mix powder.

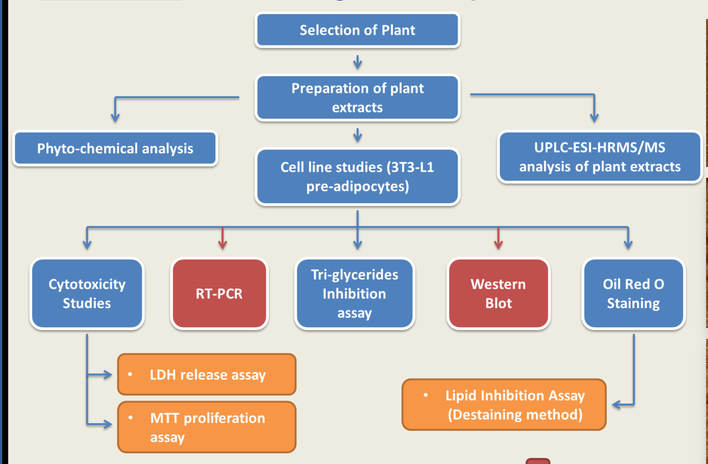
Brassica oleracea (BO)

Moringa oleifera (MO)

Ocimum basilicum (OB)

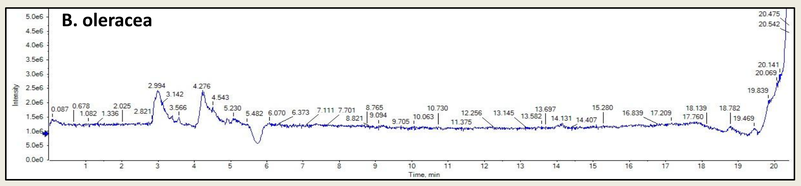
METHODOLOGY

***Flow-chart 3*: Overall design of the experiment**



- Required plant materials were purchased from Sabala Agro Product pvt. ltd. Bangalore in February 2017.
- Ethanol extracts of these plants were analysed for phytochemicals through analytical methods. (total phenols phenols, Flavanoids and anti-oxidants.)
- UPLC-HRMS/MS in +ve ion mode was utilised for identifying some major bioactive compounds present in these extracts.
- Further, these extracts were evaluated on the cultured 3T3-L1 pre- adipocyte cell line model at different concentrations against cytotoxicity and anti- adipogenic properties.
- For the cell line studies Curcumin was selected as a standard drug against obesity (Ref. Bradley S. *et. al.*).
- Number of assays were performed to prove the hypothesis.
- A functional anti-obesity soup mix incorporated with these extracts was developed.

***UPLC-HRMS/MS*: TIC + TOF MS (mass range 100-2000)**



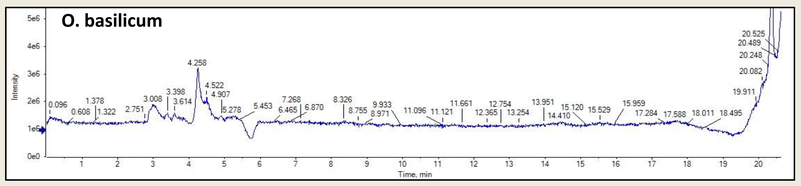
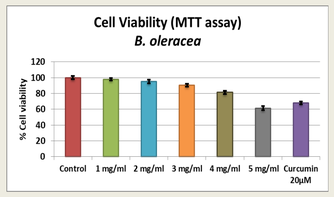


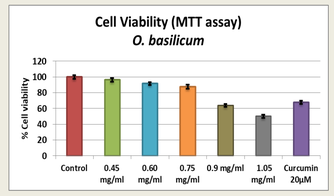
***Table 1*: Phytochemical analysis of Ethanol extracts**

Analysis	*OB* Extract	*BO* Extract	*MO* Extract
Yield (%)	20.8 ± 0.88	23.1 ± 0.93	24.9 ± 0.89
DPPH free radical scavenging activity (EC 50 µg/ml)	198.92 ± 8.04	953.41 ± 24.32	181.25 ± 7.96
Total Phenolic content (mg FAE/g)	45.69 ± 2.93	23.86 ± 0.81	65.75 ± 5.94
Total Flavonoid Content (mg QE/g)	210 ± 5.43	116 ± 4.23	232 ± 16.3

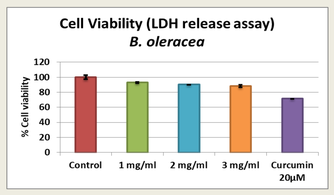
RESULTS

***Graph 1*: Cell viability assay (MTT proliferation assay)**





***Graph 2*: Cell viability assay (LDH release assay)**



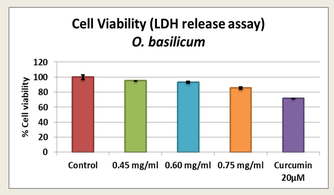
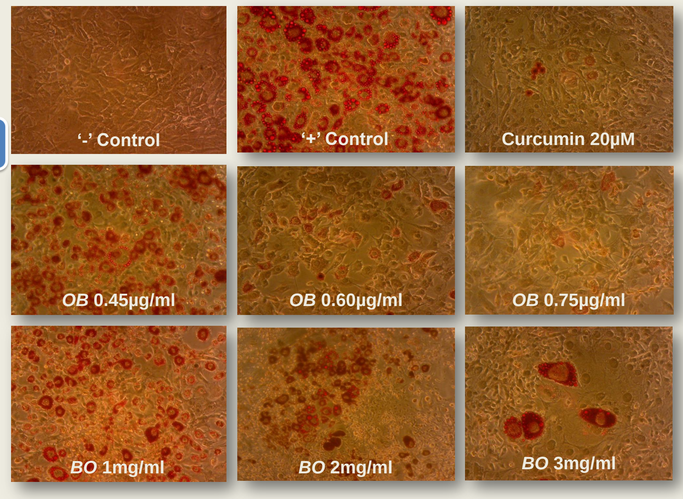
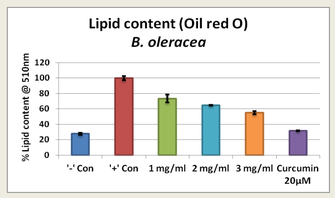
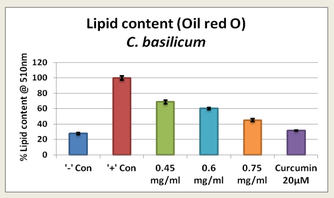


***Figure 1*: Oil Red O staining – Lipid droplets**

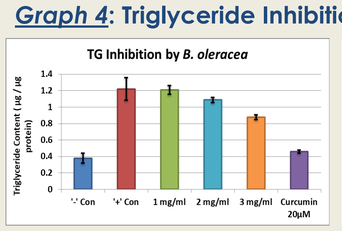


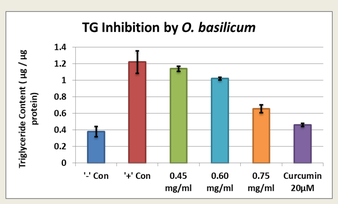
***Graph 3*: Oil Red O staining – Lipid Inhibition @ 510nm**





***Graph 4*: Triglyceride Inhibition**





Probable bioactive compounds

Compound	RT (min)	$[M+H]^+$
Vanillic acid	5.30	168.7
trans-Caffeic acid	5.55	180.6
cis-p-Coumaric acid	7.50	164.5
cis-Ferulic acid	8.12	194.8
cis-Sinapic acid	8.30	224.8

Compound	RT (min)	$[M+H]^+$
Sinapic acid	2.72	225.1
Rosmarinic acid	3.83	361.2
Methyl eugenol	6.357	179.1
Apigenin	12.356	271.02
Nepetoidin A	17.336	315.03

DISCUSSION

- Total phenolic content, flavonoid content and antioxidant content were found to be highest in *MO* followed by *OB* and *BO*.
- UPLC-HRMS/MS analysis helped in finding probable bioactive compounds in the extracts.
- These extracts exhibited anti- adipogenic effect and inhibited the triglyceride accumulation in 3T3-L1 adipocytes, dose-dependently.
- *BO* inhibited 28% triglyceride accumulation with respect to control at a conc. of 3 mg/ml while *OB* inhibited 46% at 0.75 mg/ml conc. without showing significant cytotoxicity.

PRODUCT DEVELOPMENT

***Flow-chart 4*: Product development stages**

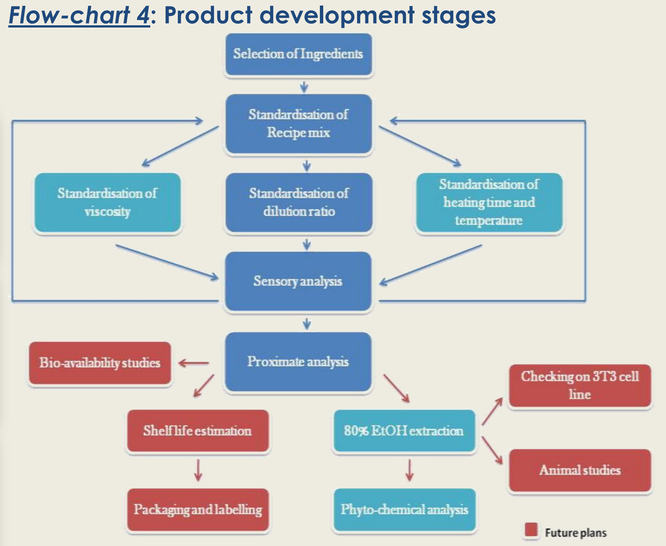


***Table 2*: Analysis of Soup mix powder**

Proximate analysis (per 10g of powder)	
Energy (kcal)	32.12 ± 1.84
Protein (g)	1.54 ± 0.14
Carbohydrate (g)	5.06 ± 0.27
of which Sucrose (g)	0.5 ± 0.02
Total Fat (g)	0.48 ± 0.02
Fibre (g)	0.63 ± 0.01
Moisture (g)	0.7 ± 0.02
Total Ash (g)	1.56 ± 0.08
Minerals (g)	1.45 ± 0.06

Vitamin analysis by HPLC (per 10g of powder)	
Vitamin C (mg)	2.67 ± 0.03
Vitamin B1 (mg)	2.00 ± 0.011
Vitamin B2 (mg)	0.53 ± 0.02
Vitamin B5 (mg)	0.34 ± 0.07
Vitamin B6 (mg)	0.35 ± 0.032
Vitamin B9 (mg)	0.29 ± 0.025

***Table 3*: Phytochemical analysis of Soup mix powder**

Analysis	EtOH Extract	MeOH Extract
Yield (%)	21.81 ± 0.8	24.1 ± 0.52
DPPH free radical scavenging activity (EC 50 µg/ml)	223.55 ± 9.42	149.18 ± 7.86
Total Phenolic content (mg FAE/g)	38.22 ± 4.5	44.48 ± 3
Total Flavonoid Content (mg QE/g)	192.6 ± 2.5	215.7 ± 4.6

Soup mix powder

2 minutes boiling (10g in 150 ml water)

Reconstituted soup

The final product showed a significant bulk of bioactive compounds and also contained a large amount of proteins (~15%) and crude fibre (~6%).

CONCLUSIONS

Present study claims that *OB* and *BO* extracts have many bioactive compounds that may be crucial against adiposity. Further investigation may substantiate the potential use of these extracts as functional foods against obesity.