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RESEARCH ARTICLE
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ANTIOBESITY ACTIVITY OF MARINE ALGAE *Turbinaria ornate*

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Abstract
Seaweeds, are plant-like organisms that generally live attached to rock or other hard substrata in coastal areas.The brown colour of the algae results from the dominance of the xanthophyll pigment fucoxanthin. Brown algae (Phaeophyceae) is a genus of *Turbinaria* found in tropical marine waters, which grow son rocky substrates.
Turbinaria ornata species are consumed by herbivorous fishes and echinoids in tropical areas, it has a low level of phenolics and tannins. It is widely distributed in tropical and subtropical areas of central, western Pacific and Indian Ocean. *Turbinaria* belongs to the class- Anthozoa, Order-Scleractinia, family-Dendrophylliidae, Genus- *Turbinaria.* Different extracts of the *turbinaria ornata* shows the presence of phytoconstituents like alkaloids, terpenes, phenols, tannins, saponins, flavonoids, quinones, proteins, sugars, carbohydrates, alkaloids, coumarines, steroids terpenoids and cardiac glycosides, they exhibit pharmacological activities like antipyretic, antimicrobial, antidiabetic,antioxidant, hepatoprotective, antifungal, antiulcer, antitumor, anticoagulant, antibacterial. further *turbinariaornata* posses phytoconstituent like phenols and flavanoids which posses pharmacological activity like antiobesity, antidiabetic, antiviral and cytotoxicity.

Keywords: Seaweeds, Phaeophyceae, *Turbinariaornata*, antifungal, terpenoid, alkaloids

INTRODUCTION

Obesity is the state of excess body fat stores, which should be distinguished from overweight (i.e., excess body weight relative to a person's height).'obesity' may be defined as an illness where the health (and hence life expectancy) is adversely affected by excess body fat. The generally accepted benchmark is the *body mass index* (BMI). The BMI is expressed as W/h^2, where W = body weight (in kg), h = height (in metres). Although it is not a perfect index (e.g. it does not distinguish between fat and lean mass), the BMI is generally well correlated with other measurements of body fat, and it is widely employed in obesity studies. While there are problems in defining a 'healthy' weight for a particular population, the World Health Organization (WHO) classifies people with a BMI of < 18.5 kg/m^2 as 'underweight', and those with a BMI of 18.5-24.9 kg/m^2 as of 'acceptable' or 'normal' weight. A BMI in the range of 25.0-29.9 kg/m^2 signifies 'grade 1 overweight'. If it is between 30.0 and 39.9 kg/m^2, the patient is deemed to be obese or 'grade 2 overweight', while those with a BMI of > 40 kg/m^2 are said to be 'grade 3 overweight' or *morbidly obese*. Childhood obesity is more difficult to assess.BMI obviously depends on the overall energy balance, another operational definition of obesity would be that it is a multifactorial disorder of energy balance in which calorie intake over the long term exceeds energy output.(1)

MATERIALS AND METHODS

Algae

Turbinaria ornata was collected from Erwadi in gulf of Mannar ,Ramanthpuram district, Tamil Nadu India on October 10, 2016. This was authentified by Dr. K. Eswaran (PrinicipalScientist),CSIR - CSMCRI MARINE ALGAL REASERCH STATION ,Mandapam Camp-623519,Tamilnadu. Adhered sand and salts were removed from the algae by washing withseawater and the algae was transported to the laboratory of Pharmacology, I.T.S college of Pharmacy, Muradnagar, Ghaziabad.

Animals

Wistar rats of either sex (150-200 g) were used for experimental purpose. The animals were housed in hygienic cages (6 rats / cage) under standard conditions of temperature $(25\pm2)^0$C, relative humidity (45±20) % and (light) 12h: (dark) 12h cycle. The rats were fed with standard pellet diet (Amrut feeds, Chakan) and water . The animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days prior to the experiments. The Formalin experimental design and research plan along with animals handling and disposal procedure were approved by Institutional Animal Ethical Committee. Registration no1044/PO/Re/S/07/CPCSEA,27 feb,2007, Project proposal no. ITS/07/IAEC/2013

Chemicals

Atrovastin, cholesterol, glucose, serum estimation commercial kit, Ellman reagent (5,5' dithiobis-1,2 nitro benzoic acid), Sulfosalicyclic acid, Eosin stain, Xylene, 100% methanol, Paraffin wax, Hematoxylin, NaCl, KCl, Potassium Dihydrogen Phosphate, Ethyl Alcohol, Formaldehyde (40%), Thymol Crystals, Magnesium Sulphate, Sodium bicarbonate, DPX, Scott reagent, Thio Barbituric Acid (TBA), EDTA, Sodium Dodecyl Sulpfate (SDS), Acetic Acid, Phosphate Buffer, Distilled Water, Glacial acetic acid, High fat diet (33% standard chow, 33% Nestlé®, condensed milk, 7% saccharine and 27% water.

EXTRACTION AND PHYTOCHEMICAL INVESTIGATION

Drying of Algae

Turbinaria ornata was shade dried at room temperature and reduced to a coarse powder with the help of the grinder, 1000 gm of the *turbinaria ornata* coarsely powderd obtained after drying ,before extraction powdered material was passed through a sieve to assure easy successive cold maceration.

Extraction Procedure

The plants of *Turbinaria ornate* was dried in shade and powdered. Dried algal powder (1000 g) was extracted with n- Hexane, three times each by cold maceration at room temperature. In cold maceration whole powdered plant drug is kept in contact with the solvent in a stoppered container for 48-72 hours with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs by Tiwari P. *et al*. (2011). For extraction, the powder was taken in round bottom flask and macerated for72-48 hours at room temperature with all material defatted completely by Handa, S. S., *et al*. (2008).The algae was extracted with three solvents with increasing polarity in order of cyclohexane, ethyl acetate and methanol respectively at the room temperature three times each. The extracts thus obtained were concentrated by distilling off the solvent under reduced pressure by using Rota vacuum Evaporator (Buchi, Germany) Suffness M., and Douros J., (1978).The defatted marc thus obtained was air dried and then the percentage yields of all three extracts i.e. n-hexane, ethyl acetate and methanol of *Turbinariaornata* was calculated .

Extraction with cyclohexane: successive cold maceratiom

1000 gm of powdered algae was cold macerated thricely for 72 hr with the cyclohexane ,for the first time the extract was macerated with 800 ml cyclohexane and then filtered, again for the second time extract was macerated with 800 ml cyclohexanne and then filtered ,same procedure was to be

followed for the third timeand the filtered extract were dried and the dried obtained extract yield was 2.4 gm.percentage yield was determined using following formula
% yield= (weight of extract in gram /1000) × 100

Extraction with ethyl acetate : successive cold maceratiom
1000 gm of powdered algae was cold macerated thricely for 72 hr with the ethyl acetate ,for the first time the extract was macerated with 800 ml ethyl acetateand then filterd, again for the second time extract was macerated with 700 mlethyl acetate and then filterd ,same procedure was to be followed in the third time and the filterd extract were dried and the dried obtained extract yield was 3.97gm.percentage yield was determined using following formula % yield= (weight of extract in gram /1000) × 100

Extraction with methanol: successive cold maceratiom
1000 gm of powdered algae was cold macerated thricely for 72 hr with the methanol,for the first time the extract was macerated with 800 ml methanol and then filtered, again for the second time extract was macerated with 700 mlmethanol and then filtered ,same procedure was to be followed in the third time and the filtered extract were dried and the dried obtained extract yield was 3.4 gm.percentage yield was determined using following formula % yield= (weight of extract in gram /1000) × 100.

Phytochemical Analysis of *Turbinaria ornata* Extracts
The extract were subjected for phytochemical investigations by qualitative chemical test.[(2)]Different extracts of the *turbinariaornata* shows the presence of phytoconstituents like alkaloids, terpenes, phenols, tannins, saponins, flavonoids, quinones, proteins, sugars, carbohydrates, alkaloids, coumarines, steroids terpenoids and cardiac glycosides.

ACUTE TOXICITY STUDIES
The acute toxicity was performed according to OECD guidelines (OECD 423, 2001). The selected wistar rats were used for toxicity studies. The animals were divided into four groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded dose like 1000, 2000 and 4000 mg/kg Body Wt. Immediately, after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24 h, and daily up to 14 days for any behavioral change or mortality. [(3)]

Experimental design
Thirty Wistar rats were divided into six groups, five in each group.
(I) Group: This group were treated as control group.
(II) Group: In this group Wistar rats were feed with a High-fat diet for a period of 6 weeks.[(4)]
(III) Group: In this group atrovastatin were given 3 mg/kg/day; orally by gavage for a period of 21 days along with High fat diet in rats for a period of 6 weeks [(5)]
(IV) Group: The animal of this group were given methanolic extract 4000mg/kg/day; orally for 6 weeks along with High fat diet in rat for a period of 6 weeks.
(V) Group: In this group cyclohexane extract were given at a dose 4000 mg/kg/day; orally for a period of 6 weeks after induction of obesity with High fat diet in rats for a period of 6 weeks
(VI) Group: In this group methanolic extract were given at a dose 4000 mg/kg/day; orally for a period of 6 weeks after induction of obesity with High fat diet in rats for a period of 6 weeks.

Biochemical parameters [(6)]
At end of treatment period blood samples were collected from all the groups of the animals through the orbital sinus without the use of anti-coagulant. The blood sample was Centrifuged using centrifuge at 2000 rpm for 30 min to get serum for study of various biochemical parameters. The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit.

Estimation of cholesterol

Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterolis oxidized to form hydrogen peroxide which further reacts with phenol and 4-amino antipyrine by the catalytic action of peroxidise to form a red coloured quinineimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

Cholesterol Esterase → Cholesterol Esters +H2O Cholesterol +Fatty acids.

Cholesterol oxidase →Cholesterol + O2 Cholestenone + H2O.

H2O2 + 4aminoantipyrine + Phenol Red →Quinoneimine dye + H2O.

Pipette out 1.0ml of working reagent into a clean dry test tube and add 0.01ml of sample to it mix well and incubate at 370 C for 5 min and aspirate.

Estimation of Triglycerides

Triglycerides are first hydrolyzed by lipoproteinlipase to glycerol and free fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate(ATP) forming glycerol-1-phosphate (G-1-P) andadenosine-5-diphosphate (ADP) in the reaction catalyzed by glycerol kinase (GK). G-1-P is thenoxidized by glycerol phosphate oxidase (GPO) to dihydroxy acetone phosphate (DAP) and hydrogenperoxide (H2O2). Peroxidase (POD) catalyzes the coupling of H2O2 with 4-aminoantipyrine (4-AAP)and sodium N-ethyl-N-(3-sulfopropyl) manisidine(ESPA) to produce a quinoneimine dye Triglycerides → Glycerol + Fatty acids

GK: Glycerol + ATP → G-1-P + ADP

GPO: G-1-P + O2 → DAP + H2O2

POD: H2O2 + 4-AAP + ESPA→Quinoneimine dye + H2O.

HDL

The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing assay and only HDL-chol is detected under the assay conditions. The method uses sulfated alphacyclodextrinnin the presence of Mg+2, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement.

ApoB containing lipoproteins + α-cyclodextrin +Mg+2 + dextran SO4 → soluble nonreactive complexes with apoB-containing lipoproteins.

PEG-cholesteryl esterase HDL-cholesteryl esters----------→HDL unesterified cholesterol + fatty acid.

PEG-cholesterol oxidase Unesterifiedchol + O2----------→ cholestenone +H2O2.

H2O2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine+ H2O + H+ peroxidase----------→qunoneimine dye +H2O

Estimation of LDL

Using data obtained above the low density lipoprotein cholesterol levels were calculated using empirical formula of friede waid Serum Low density lipoprotein =total cholesterol- High density lipoprotein Triglycerides

Measure the physical parameters

Percentage change in body weight:

1. Body wt. of individual animal were taken for each group and record were maintained.
2. Body wt. Were taken daily from the starting day of the study till the last dosing was done before sacrificing the animal.
3. If death of any animal occurs in between the study time, its weight were taken.
4. Any change in the body wt. of the animal were record.

Histopathological examination of fat pad:

Isolation of fat pads

Three regions of adipose tissue were carefully dissect:

1. The periovarian fat, ovaries were taken out by gentle squeezing from the peripheral fat and then by horizontal cut from all sides fat was isolated; care has been taken that too much traction was avoided on ovaries and fat.
2. The retroperitoneal, by first separating the perirenal fat and then dissecting the retroperitoneal pad in to.
3. The mesenteric, all fat found along the mesentery starting at the lesser curvature of the stomach and ending at the sigmoid colon was considered mesenteric fat.

The periovarian fat was selected for histological study. The periovarian fat of each group were excised and rinsed in 0.9% saline blotted dry of saline and excess blood. They were fixed in 12% formalin for 24 h. The tissues, after fixation, were washed in water to remove excess fixative. Washed tissues were then dehydrated through a graded series of ethyl alcohol, cleared with xylene and embedded in paraffin wax. Sections were cut at 3 μm with microtone blade and mounted on clean glass slide. The sections were routinely stained with hemotoxylin and eosin. The stained slides were observed (×200) in research microscope and photographed .[7]

Statistical analysis:

Results were shown as Mean ± SEM for each group. Statistical analysis were performed by using Sigma Stat and Sigma Plot statistical software. Significance of difference between two groups were evaluated. For multiple comparisons, one-way analysis of variance (ANOVA) were used. In case ANOVA were shown significant difference, post hoc analysis were performed. $P < 0.01$wereconsidered being statistically significant.

RESULTS AND DISCUSSSION

PERCENTAGE YIELDS OF EXTRACTS

S.No	Extracts	Amount of extract (in gms)	Percentage yield
1	Hexane extract of *Turbinariaornata*	2.4	0.24%
2	Ethyl acetate extract of *Turbinariaornata*	3.97	0.397 %
3	Methanolic extract of *Turbinariaornata*	12	1.2 %

ACUTE TOXICITY STUDIES

Doses given to different groups for acute toxicity studies

S. no.	methanol extract of *Turbinariaornata*	Ethyl acetate extract of *Turbinariaornata*	Cyclohexane extract of*Turbinariaornata*
1	1000mg/kg	1000mg/kg	1000mg/kg
2	2000mg/kg	2000mg/kg	2000mg/kg
3	4000mg/kg	4000mg/kg	4000mg/kg

Hence the selected dose for the study was **400 mg/kg** of each of the three extracts**.**

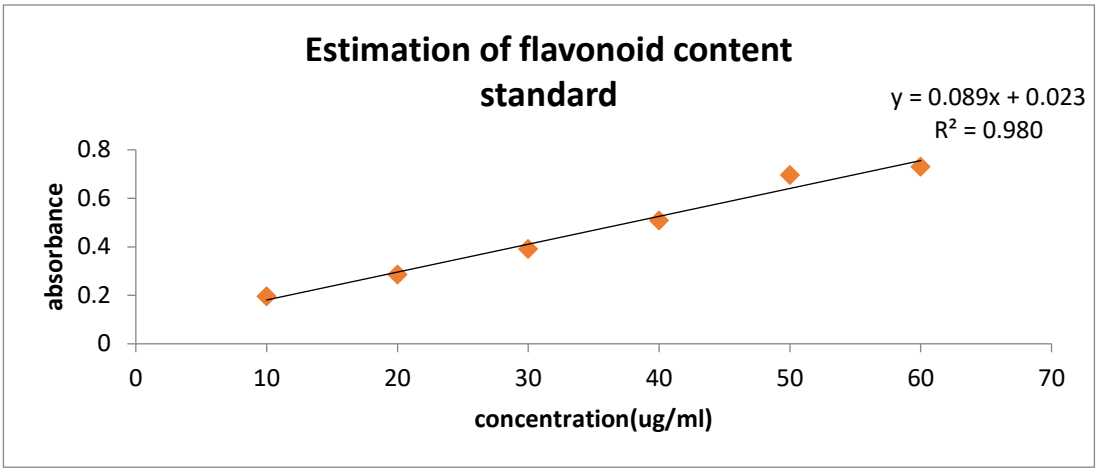
Phytochemical Analysis of *Turbinariaornata* Extracts

S. no.	PHYTOCONSTITUENTS PRESENT	Hexane extract of *Turbinariaornata*	Ethyl acetate extract of *Turbinariaornata*	Methanol extract of *Turbinariaornata*
1	Alkaloids	(+)	(+)	(+)
2	Cardiac glycosides	(+)	(+)	(+)
3	Tannins	(+)	(+)	(++)
5	Reducing Sugars	(--)	(--)	(+)
6	Anthraquinones	(--)	(--)	(+)
7	Steroids	(--)	(--)	(+)
8	Proteins	(--)	(+)	(+)
9	Flavonoids	(+)	(+)	(+)
10	Saponins	(+)	(+)	(+)
11	Phenols	(+)	(+)	(+)

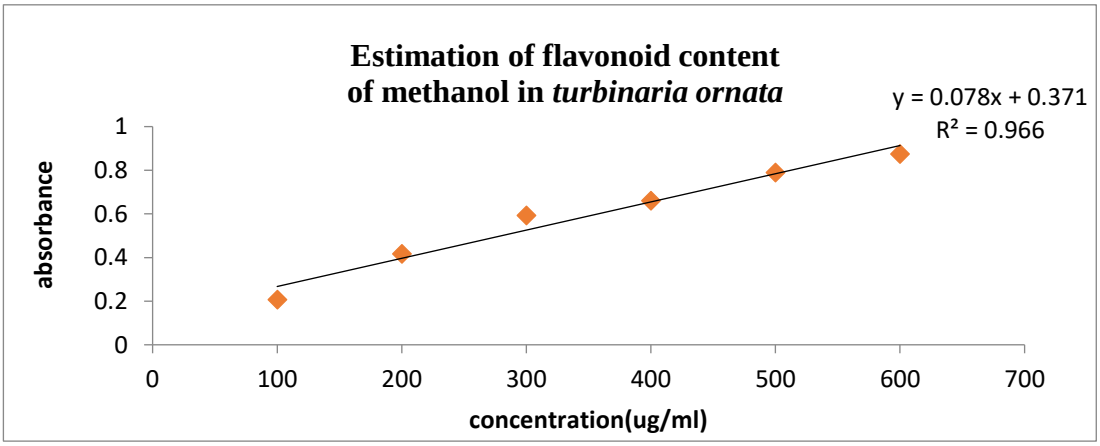
(+) indicates presence (-) indicates absence

TOTAL FLAVONOID CONTENT

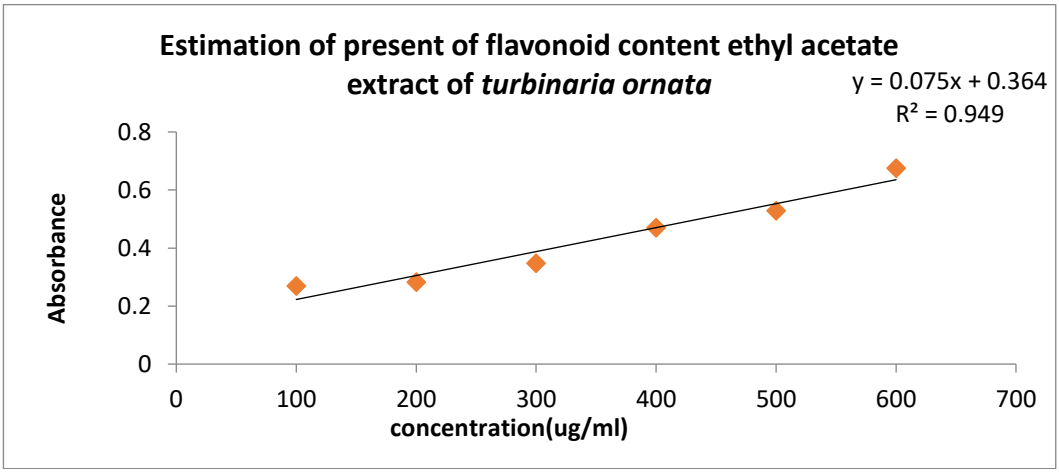
S. No.	Concentration (µg/ml)	Absorbance of extracts		
		methanol	Ethyl acetate	Cyclohexane
1	100	0.207±0.057	0.269±0.02	0.194±0.06
2	200	0.417±0.05	0.283±0.055	0.233±0.050
	300	0.537±0.017	0.348±0.013	0.238±0.064
4	400	0.660±0.02	0.470±0.023	0.366±0.021
5	500	0.790±0.019	0.529±0.019	0.464±0.086
6	600	0.874±0.043	0.774±0.043	0.559±0.032



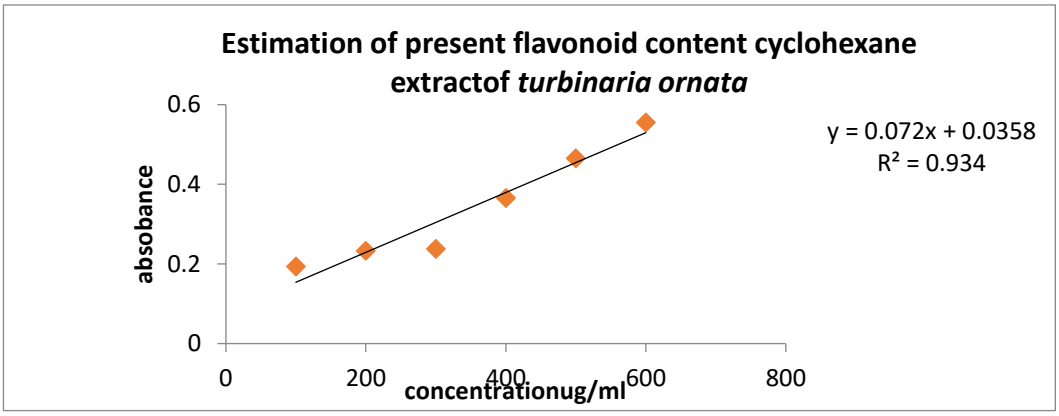
standard curve of Quercetin



Total flavonoid content in methanol extract of *Turbinaria ornata*(mg/g)



Total flavonoid content in ethyl acetatel extract of *Turbinaria ornata*(mg/g)



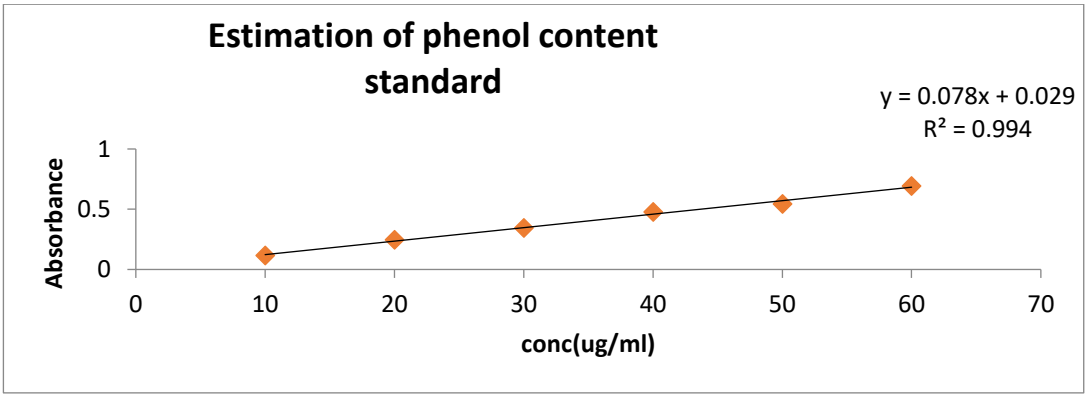
Total flavonoid content in cyclohexane extract of *Turbinaria ornata*(mg/g)

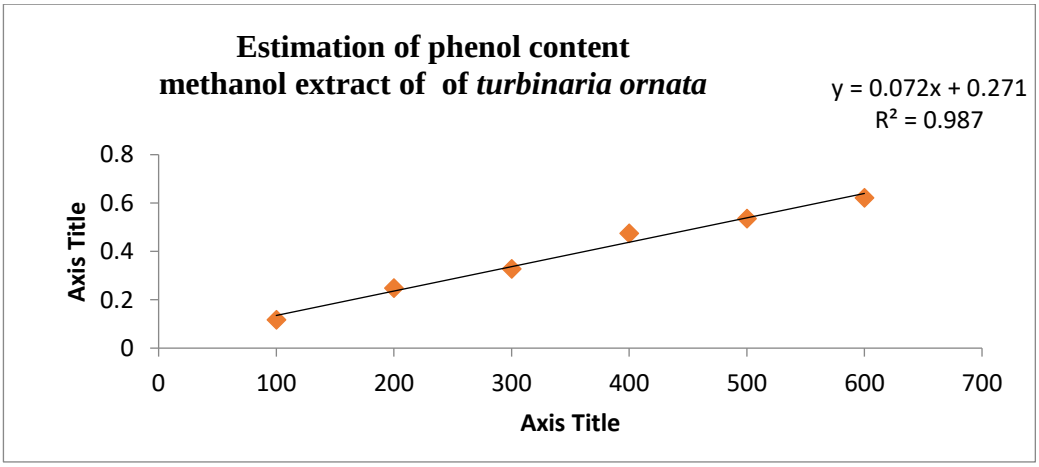
S.No	*Turbinariaornata*extracts	Total flavonoid content (mg/g
1	Methanol extract of *Turbinariaornata*	31.6
2	Ethyl acetate extract of *Turbinariaornata*	24.3
3	Cyclohexane extract of *Turbinariaornata*	19.7

Total flavonoid content in different extracts of *Turbinariaornata*

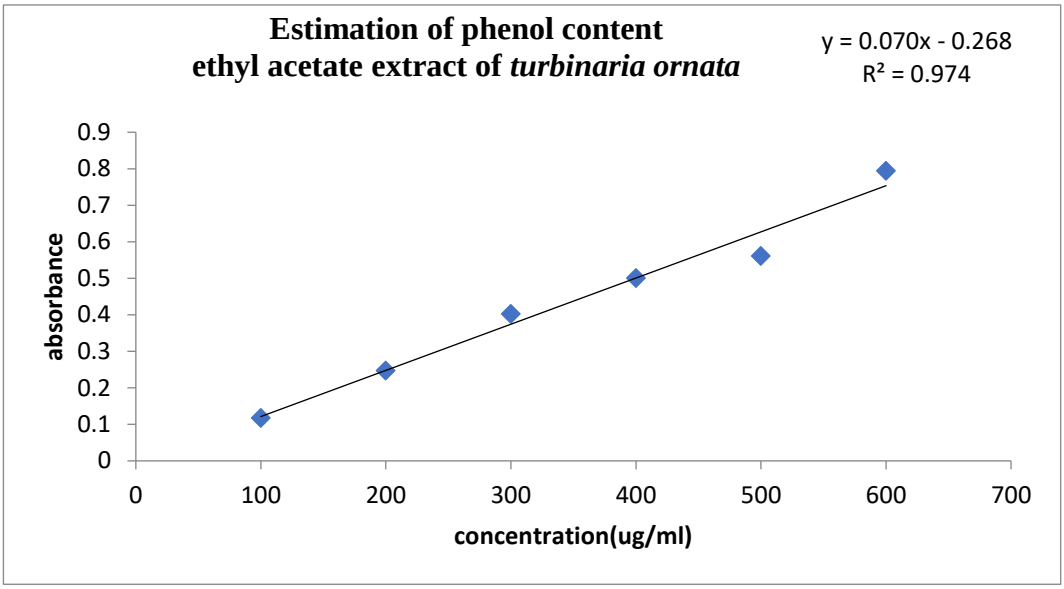
Total phenolic content estimation

S. No.	Concentration (µg/ml)	Absorbance of standard (Gallic acid)
1	**20**	0.115±0.25
2	**40**	0.245±0.65
3	**60**	0.345±0.05
4	**80**	0.476±0.04
5	**100**	0.693±0.06

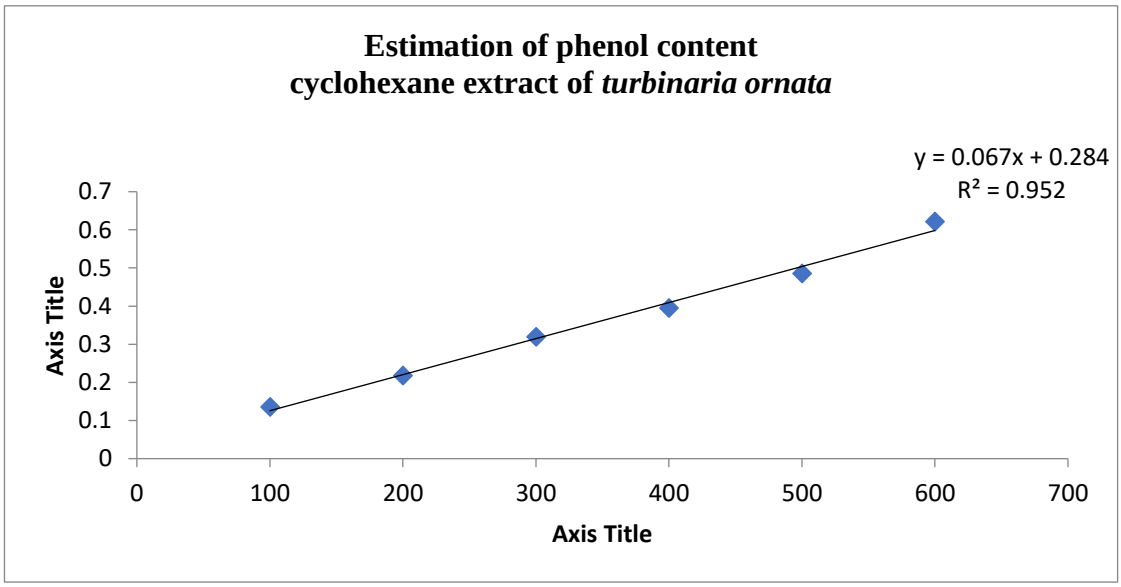




Total phenolic content in methanol extract of *Turbinaria ornata*(mg/g)



Total phenolic content in ethyl acetate extract of *Turbinaria ornata*(mg/g)



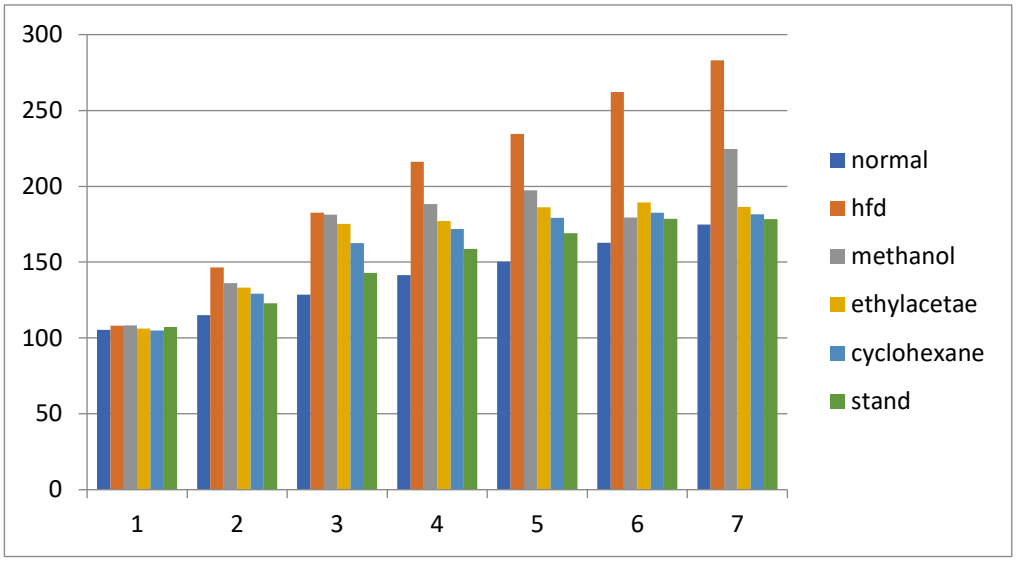
Total phenolic content in cyclohexane extract of *Turbinaria ornata*(mg/g)

S.No	*Turbinariaornata*extracts	Total phenolic content (mg/g)
1	Methanol extract of *Turbinariaornata*	40.3
2	Ethyl acetate extract of *Turbinariaornata*	30
3	Cyclohexane extract of *Turbinariaornata*	23.16

ANTIOBESITY ACTIVITY

S No	Weeks	Normal	POSITIVE CONTROL	Methanol	Ethyl acetate	Cyclohexane	Standard
1	0	150.28±1.64	170.07±1.90	160.12±1.8**	165.1±1.79*	185.13±1.58*	180.24±1.81**
2	1	165.10±2.15	180.54±2.93	178.16±2.9**	168.25±2.56*	190.25±3.56*	185.91±2.55**
3	2	170.55±2.25	190.55±3.38	185.28±3.4**	175.28±2.72*	192.34±2.56*	190.02±3.22**
4	3	180.40±2.72	195.12±3.70	190.29±3.3**	195.07±2.98*	195.57±2.27*	195.77±3.27**
5	4	185.31±2.79	235.44±3.75	200.44±3.6**	200.25±3.45*	200.59±2.48*	200.17±3.48**
6	5	190.85±2.81	262±3.86	195.44±2.9**	210.39±3.51*	215.61±2.72*	198.62±3.72**

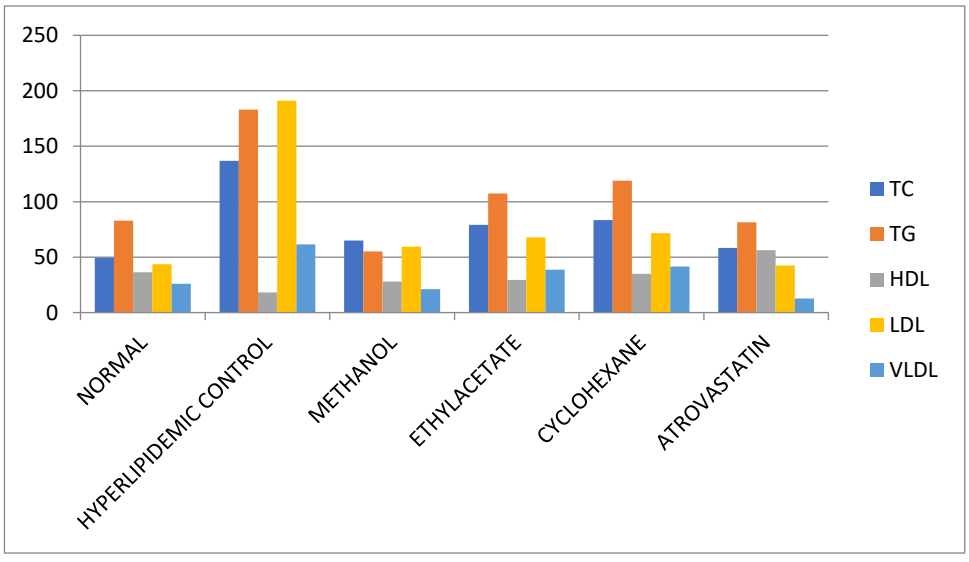
All Values were expressed as mean ± SD (n = 5) , *p<0.001 when compared to control group = **p<0.002 when compared to standard (One way ANOVA followed by Dunnett's test).



Effect of different *extracts* of *Turbinaria ornate* on body weights in high fat diet induced obese wistar rats

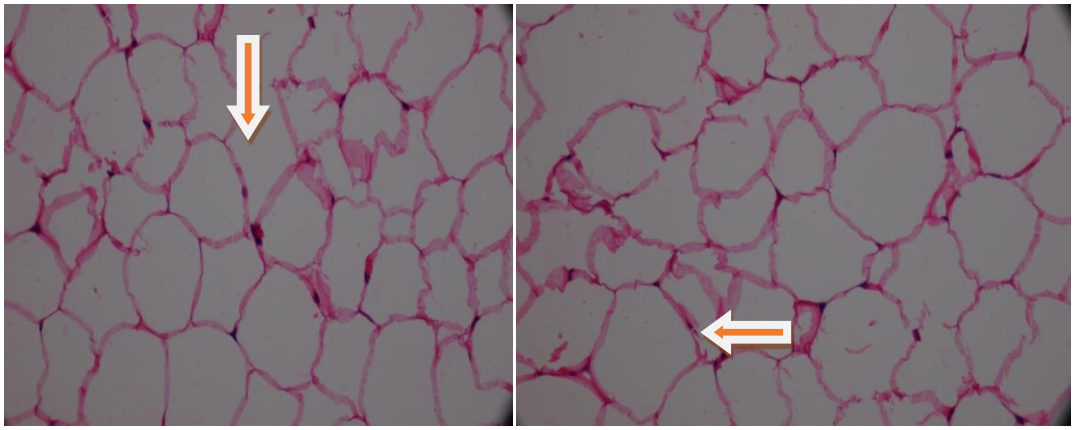
S NO	GROUPS	TC	TG	HDL	LDL	VLDL
1	NORMAL	83.46±1.47	80.86±2.12	36.51±5	39.6 ±4	25.91±3.9
2	POSITIVECONTROL	195±8.2	125.97±8*	78.24±3.8*	85.25±5.7**	61.43±3.9**
3	METHANOL	125±3.9	85.21±8.2	22.9±6.3**	59.55±4.8*	26.2±6.1
4	ETHYLACETATE	165.18±8.7	94.4±6.4	29.44±3.9	67.78±6.1	35.8 ±4.9
5	CYCLOHEXANE	170.32±5.9	100±5.6	34.89±4.2**	71.46±3.9*	41.68±3.5**
6	ATROVASTATIN	90.18±3.8	81.4±4.9*	32.43±2.7**	41.34±3.8**	22.8±4**

All Values were expressed as mean ± SD (n = 5), *p<0.001 when compared to control group = **p<0.002 when compared to standard (One way ANOVA followed by Dunnett's test).

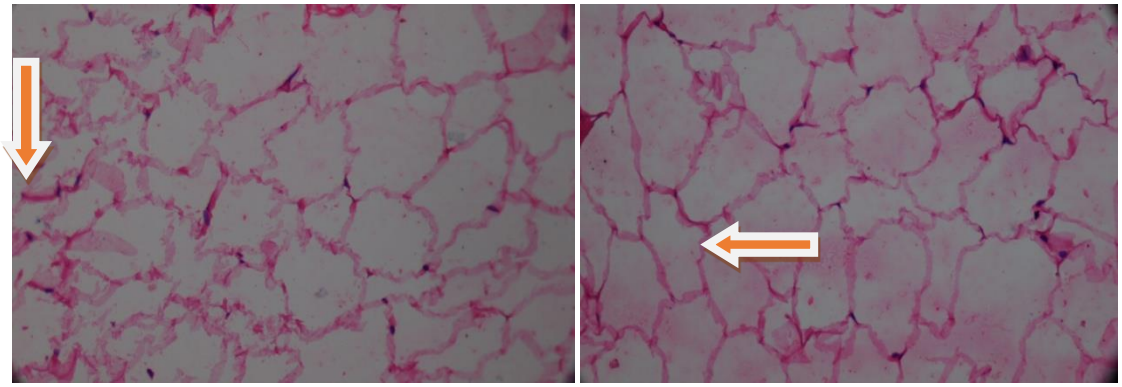


Histogram representing effect of different extracts of *Turbinaria ornaata* on serum lipid levels against high fat diet induced obesity.

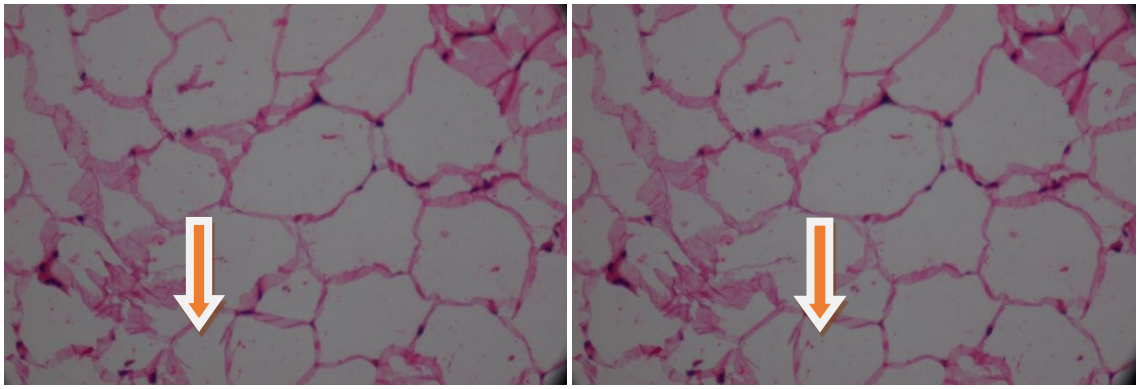
MICROSCOPIC EVALUATION BY HISTOPATHOLOGY
ANTIOBESITY ACTIVITY
Histopathological Changes In The Adipocytes



(Control group of*Turbinariaornata*), the adipocytes of the normal shows destruction in cell walls and arrow shows shrinkage of adipocytes
(Standard group of*Turbinariaornata*) ,the adipocytes shows the reduction in size , shrinkage as well as arrow shows destruction of adipocytes



(Positive control group of*Turbinariaornata*) ,the arrow indicates the obese adipocytes after receiving the high fat diet
(Methanol extract of *turbinaria*ornata)), after the administration of the methanolic extracts arrow shows the destruction in cell wall which affects the integrity of cell



Cyclohexane extract of *turbinaria*ornata)),After administration of the cyclohexane extracts it indicates the shrinkage of cell as wall as the arrow shows destruction of cell wall , this destruction can leads to the leakeage of the cell contents which cause apoptosis of the adipocytes.
(ethyl acetateextract of *turbinaria*ornata))After administration of ethyl acetate the arrow indicates less shrinkage and less destruction of adipocytes

CONCLUSIONS

The extracts of *Turbinariaornata*like methanol , cyclohexane and ethyl acetate were screened for the presence of the phytoconstituents both qualitatively and quantitatively. Phytochemical screening showed the presence of saponins,glycosides, phenols, flavonoids, alkaloids and tannins,

carbohydrates, steroids. All the extracts were successively cold macerated for 72 hours.Theflavonoid content in the methanolic extract was calculated to be 31.6 mg/g Quercetin equivalent, ethyl acetate contained 24.3 mg/g Quercetin equivalent while thecyclohexane extract contained 19.7 mg/g Quercetin equivalent.The phenolic content was estimated to be 40.3mg/g gallic acidequivalent in methanolic extract of, ethyl acetate extract of*Turbinariaornata*contained 30 mg/g gallic acid equivalent while the cyclohexaneextract of *Turbinariaornata*contained 23.16 mg/g gallic acid equivalent phenoliccontent On acute oral toxicity the extract was found to be safe up to 4000 mg/kgand thus the 1/10th of the dose 400mg/kg was selected for the studies.

The present examination was carried out to scientifically evaluate the cyclohexane, ethyl acetate and methanolic extracts of *Turbinariaornata*for the antiobesity model. Group1 will be treated as control group, group 2 Wistar rats will feed a High-fat diet for a period of 6 weeks, group 3 atrovastatin will be given 3 mg/kg/day; orally by e for a period of 21 days along with High fat diet in rats for a period of 6 weeks The animal of the group 4 will be given methanolic extract 3000 mg/kg/day; orally for 6weeks along with High fat diet in rat for a period of 6 weeks. In group 5 cyclocyclohexane extract will be given at a dose 3000 mg/kg/day; orally for a period of 6 weeks **,** group 6 methanolic extract will be given at a dose 3000 mg/kg/day; orally for a period of 6 weeks after induction of obesity with High fat diet in rats for a period of 6 weeks , parametersAll the three extracts were found to significant at $p<0.001$ and inhibit the antiobesity activity. The methanolic extract shows the highest antiobesity activity followed by the ethyl acetate andcyclocyclohexane extracts against the atrovastatin as the standard which showed the highest antiobesity activity.

REFERENCES

1. Barbara G. Wells, Joseph T. Diprio ,Cecily V. Diprio : Phamacoherapy handbook ; seventh edition, Jaypee brothers medical publishers (p) ltd. ,663.
2. Kokate CK, Purohit AP, Gokhale SB, Pharmacognosy. Pune, India: Niraliprakashan, 2007.
3. Health Effects Test Guidelines Acute Oral Toxicity OPPTS 870.1100 United States Office of Prevention, Pesticides and Toxic substances Environmental Protection Agency (7101).
4. Choh. H. K.; *et a*l., Antiobesity Effect of Codonopsis lanceolata in High-Calorie/High-Fat-Diet-Induced Obese Rats, *Evidence-Based Complementary and Alternative Medicine,* 2013, 2013, 9
5. Brown. M.; *et al*., Sibutramine reduces feeding, body fat and improves insulin resistance in dietary-obese male Wistar rats independently of hypothalamic neuropeptide Y, *British Journal of Pharmacology*, 2001, 132, 1898–1904.
6. Gupta. S. K, Drug Screening Methods, *Jaypee*, 2009.
7. James. K..; *et al.,* A Mouse Model of Diet-Induced Obesity and Insulin Resistance.