EVALUATION OFANTIOXIDANT, ANTICANCER AND ANTIPARKINSON'S POTENTIAL OF DCM-ME EXTRACTS OF *Dendrophthoe falcate*(L) *ANDTridax procumbens*(L) PLANTS

Vishwas C. Bhagat[1]*, Manish Kondawar[2], Deepak P. Kardile[1], Pravin B. Awate[2], Santosh R. Tarke[3], Rahul A.Sonawane[1]

[1]Department of Pharmaceutical Chemistry, Rajgad Dnyanpeeth's College of Pharmacy, Bhor (Pune) Maharashtra, India- 412206.

[2]Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India- 416416.

[3]Department of Pharmacognosy, Shri Balaji Shikshan Prasarak Mandal's College of Pharmacy, Ambajogai (Beed) Maharashtra, India- 431517.

Corresponding Author: Dr. Vishwas C. Bhagat, Assistant Professor, Department of Pharmaceutical Sciences, Rajgad Dnyanpeeth's College of Pharmacy, Bhor (Pune) Maharashtra, India- 412206.
Email ID: vishumed@gmail.com

Abstract:

To evaluate antioxidant, anticancer and antiparkinson's potential of DCM-ME extracts of *Dendrophthoe Falcate* (L) *andTridax procumbens* (L) plants.DCM-MEplants extracts and their fractions were prepared according to standard procedures.Phytochemical Pharmacognostic and HPTLC study of fractions of DFDM and TPDM extractsshowspresences of secondary metabolite alkaloids, flavonoids, saponins, tannins, phenolic group, glycosides,phytosterols and terpenoids.Quantitative HPTLC study DFDM-I& TPDM-I (5µl/ml) fractions show presence of rutin at Rf value 0.54 and 0.57 having area 6580.6AU and 7469.3AU. Rhodoxanthin, astaxanthin&Squalene are isolated and identified by GC-MS from DFDM-I & TPDM-I fractions. The antioxidant activityby using reducing power assay method which shows (60.62% & 52.66% Ascorbic acid-100%), respect to phenol content found 17.44mg&8.52mg.The cytotoxic activity DFDM &TPDM extractswere studied against Cell line- HEK-293(IC_{50}< 20µg/ml &>40µg/ml), Hela (IC_{50}< 40µg/ml &>20 µg/ml), compare to Cisplatin IC_{50}-10 µg/ml. by MTT assay. Antiparkinson's activity evaluated by haloperidol induces catalepsy,DFDM (100 mg/kg groups showed significant decrease in latency period ($p<0.01$) when compared to haloperidol treated group and significant inhibition ($p<0.05$, $p<0.01$) of Clonidine induced catalepsy in the animal by DFDM &TPDM Identified and characterized Rutin, Rhodoxanthin,Astaxanthin&Squalene may responsible for antioxidant, anticancer and antiparkinson's activity. Research provides best data for development of new phytochemical as anticancer, antiParkinson's medicine which is socially benefit in future.

Keywords:*Dendrophthoe falcate, Tridax procumbens,* HPTLC; GC-MS; Cytotoxicity; antiparkinson's.

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1. INTRODUCTION

Today worldwide there is a need new medicines potent effect and less toxicity so plant origin phytomolecules are the best choices for treatment of cancer, tubercular and disorders belongs to central nervous systems. A *Dendrophthoe falcata* (L.F.) hemiparasitic plant commonly known as 'Vanda' belongs to family loranthaceae from the host plant Mangifera indica. To date *D.falcata* represents the only known mistletoe with the largest global host which is continuously and rapidly widening. The whole plant is used in indigenous system of medicine as cooling, bitter, diuretic, narcotic.[1]*Dendrophthoe falcata* possesses

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remarkable potentials as plant proved from the antibacterial, antifungal; wound protecting, antioxidant analgesic propertiesof its alcoholic extracts, [2-3]anti-fertility,[4] anticancer,[5-6]antioxidant[7-8] and antitubercular [9]Quercetin, Kaempherol, Quercetrin are the different flavonoids isolated and reported from whole plant of *D. Falcata*.[10-11]

Tridax procumbens (L). family -Compositae commonly known as 'coat buttons' because of appearance of flowers has been extensively used Ayurveda in liver disorders.[12]*Tridax* possesses significant anti-inflammatory, hepatoprotective, wound healing, antidiabetic activity and antimicrobial activity against both gram-positive and gram-negative bacteria, [13-16] anticancer.[17-19] Flavonoids isolated and reported from whole plant.[20-21]The common phytochemicals constituents reported from *Dendrophthoe falcata (L.F.*) plant such as β-amyrin acetate, β-sitostirol, Stigmasterol, kaemferol, Quercetin,[22] quercetin–3-*O*-rhamnoside, rutin, myrecitin, leucocyanidin and three biologically important cardiac glycosides such as strospeside, odoroside F and neritaloside.[23]Above literature shows that *D. falcata* is therapeutically useful in oxidative stress induced diseases.

The basic fraction from the leaves of *T. procumbens* has been identified as alkaloids, carotenoids, flavonoids(catechins and flavones), saponins, and tannins.Luteolin, glucoluteolin, β-sitosterol quercetin, β-sitosterol-3-O-β-Dxylopyranoside, and flavonoid Procumbenetin have been isolated from leaf .[20]

The aim of the present work was to evaluate antiparkinson's activity by haloperidol induces catalepsy,Clonidine induced catalepsy in the animal by DFDM & TPDM (100 mg/kg, p.o.) along with the total phenolic content, reducing power assay (in vitroantioxidant) activity of DCM-ME extracts of *D. falcata* and *T. procumbens* and anticancer activity of DFDM, TPDM extracts against Cell line-, HEK-293, Hela, by MTT assay.

2. MATERIALS AND METHODS:

2.1. Plant collection and identification:

The plant material of*Dendrophthoe falcata* (L.f.) family-loranthaceae and *Tridax procumbens (*Linn) family-asteraceae) were collected at flowering stage from local region Kapurhol Satara-Pune road (NH-4) Pune, Maharashtra (India), during September – November. Both specimens plant were identified and authenticated by Botanical Survey of India (BSI), Pune, Maharashtra (India). Voucher specimen No- VIBTRP2 and VIBDEF3 dated Reference number-BSI/WRC/TECH/2013 was deposited in the BSI herbarium.

2.2. Extraction of plant material and fractionation:

Extraction of plant material done using the soxhlet apparatus. The air dried leaves were coarsely powdered using a laboratory mill. 500 g of the plant material was extracted in dichloromethane: methanol for 48 h with manual intermittent shaking at 2 h interval. The extract was then filtered using Whatman No 1 filter papers and later concentrated in vacuumusing rotary evaporator at 40^{o}C. The dried extract thus, obtained was sterilized by overnight UV-irradiation. The sterile extract was transferred into a sterile flask & frozen in a deep freezer. The extract was stored at – 20^{0} C till bio evaluation. Fractions of DFDM & TPDM extracts were prepared by column chromatography using silica gel p.s (60-120) by using mobile phase n-Hexane : Toluene : Ethyl acetate (2: 4 : 1.3), sub fractionated by n-Hexane : Toluene : Ethyl acetate (2:4:0.8).

2.3. Animals:

Healthy adult Swiss Albino mice of both sexes were used as per experimental protocols (IAEC/ABCP/12) after consent from Institutional Animal Ethical Committee and Appasaheb Birnale College of pharmacy,

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Sangli Maharashtra, India. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. The animals (weighing 25-35g) were obtained from approved breeders and house in standard environmental conditions ambient temperature at 23 ± 2°C, with 65 ± 5 % humidity and feed with standard diet and water. The mice were destitute of food for 24 hrs before conducting tests. Each group comprises 6 mice.

2.4. Phytochemical Analysis:

The phytochemical investigation of the differentfractions of DCM-ME extracts of *D. falcata* and *T. procumbens*were performed for the detection of phytoconstituents like alkaloids (Wagner's test), flavonoids (Shinoda's test), glycosides (Legal's test), phenolics (With 5% Ferric chloride solution), sugars (Molisch's test), tannins (With 10% Lead acetate solution), saponin (Foam Test), steroids (Salkowski reaction) and terpenoids (Salkowski Test)carried out with standard protocol. [24-25]

2.5. Chromatographic Analysis:

2.5.1. HPTLC Study:

Quantitative separation of Rutin by HPTLCstudies were carried out by the method of Harborne [26] and Wagner *et al*.,. [27-28] The 0.5g Sample dissolved in 2ml Ethyl acetate.

1 mg of Rutin Standard Dissolved in 10ml Methanol (100ppm)**.**The 1 µl, 5 µl, of sample extract was applied with the help of Linomat syringe using the Linomat applicator 5. Solvent system *n*-hexane: toluene: ethyl acetate (2: 4: 1.3) HPTLC silica gel F254 (Merck). The plates were developed in a CAMAG chamber. CAMAG HPTLC densitometer (Scanner) used to measure absorbance mode at 366 nm. Data integration through the software WINCATS planar chromatography manager. The fingerprint so developed and R_f value were noted. Spots were visible without derivatization at 366 nm. A solvent system optimized for TLC study was chosen for HPTLC study.

2.5.2. GC-MS Analysis:[29]

GC-MS analysis of fractions of DFDM-Ia and TPDM-IShows finding number of probable phytoconstituents.GC-MS instrument used at Center for Food Testing, Bharti Vidyapeet, Pune, India. Agilent 7890A Gas Chromatography, Agilent 7000B Mass spectrophotometer (USA) coupled with triple quadrupole mass spectrometer detector. The GC-MS system was equipped with a DB-5MS column (30 mm× 0.25mm i.d., 0.2 micron film Filter). Carrier gas Helium is used as at a flow rate of 1.0 mL/min and a split less. In temperature programming initial temperature is 110^{0C} (hold 2 min ramped at 15 °C/min to 150 °C (hold 1 min) ramped at 10 °C/min to 280^{0C} (hold 5 min), final experiments total run time 23.66 min. The injector and detector were held at 250^{0C}. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 50–700.amu.

2.6. Pharmacological study:

2.6.1. Antioxidant activity:

2.6.1.1. Total phenols content:[30]

Total phenols content was determined using colorimetric method. 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin-Ciocalteu reagent and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample (extract). Extracts were performed in triplicates.

2.6.1.1.1. Calibration curves of Gallic acid: [30]

Accurately weighed 100 mg of gallic acid was dissolved in 100 ml of distilled water which

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gives the concentration of 1000 µg/ml. 10 ml of this solution was taken and made up to 100 ml with gallic acid which contains the concentration of 100 µg/ml. Further 10 ml of this solution was taken and made up to 100 ml with gallic acid which contains the concentration of 10 µg/ml. 1 to 10 ml were taken from this solution and made up to 10 ml to get the concentration ranges of 1 to 10 µg/ml. Calibration curve was plotted by mixing 1 ml aliquots of gallic acid solutions with 2.5 ml of Follian-Ciocalteu reagent and 2.0 ml of sodium carbonate solution (75g/l). The absorbance was measured after incubation at room temperature for 2 h at 760 nm using UV spectrophotometer, against blank solution.

2.6.1.2. Reducing power assay: [31-32]

The relative reducing activity in terms of antioxidant activity of extracts was determined by using individual extracts (5 mg) as well as its combination with equal amount of ascorbic acid The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionised water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferrocyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared FeCl3 solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 µg mL-1 extracts aliquot. Increased absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid.

2.6. Anticancer activity

2.6.1. MTT assay:[33-37]

Cell culture used for the study was procured from NCCS, Pune. The cell line were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamycin(10ug), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37ºC for 3-4 days. After 3-4 days remove the supernatant and replace MEM media with Hank's balanced solution supplemented with Gentamycin, Penicillin and Streptomycin. Incubate overnight. MTT solution preparation 10 mg in 10 ml of Hank's balanced solution

Cytotoxicity Assay -Invitro growth inhibition effect of test compound was assessed by spectrophotometrically determination of conversion of MTT into "Formazan blue" by living cells. Remove the supernatant from the plate and add fresh Hank's balanced salt solution and treated with different concentration of extract or compound 10, 20, 30 (µg/ml) appropriately diluted with DMSO. Control group contains only DMSO. After 24 hrs incubation at 37ºC in a humidified atmosphere of 5% CO_2, the medium was replaced with MTT solution (100µl, 1mg per ml in sterile Hank's balanced solution) for further 4 hr incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue' were solubalised by adding DMSO (200µl) and optical density was measured at wavelength of 490nm / 570nm. Using Cisplatin as a positive control. The result were represents the mean of three readings .The concentration at which the OD of treated cells was reduced by 50% with respect to the untreated control.

Formula:

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control}} \text{ X } 1$$

2.7. Anti-Parkinson activity:

2.7.1. Haloperidol induced catalepsy in mice (Catalepsy bar test):[38-39]

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Albino mice were divided into five groups (n = 5). Control group received saline (1 ml/kg) and Second and third groups received dose of Haloperidol (Hal.) 1 mg/kg i.p. and Levodopa 30mg/kg i.p + Hal. Fourth and fifth group received DFDM extract and TPDM extract 100 mg/kg, p.o. + Hal. Catalepsy was induced with haloperidol. Latency period at different time point intervals (0, 60, 120, 180, 240 mins) after haloperidol administration were added and expressed as average latency period. A cut off time of 180 sec was applied. Catalepsy was measured by means of a standard bar test, as the time during which the animal maintained an imposed position with both front limbs raised and resting on wooden bar above the surface

2.7.2. Clonidine induced catalepsy (Wood Block test):[40-41]

Albino mice were divided into four groups (n = 5). Control group received saline (10ml/kg) and Second and third groups received single dose of DFDM extract of *Dendrophthoe falcate* (L.F) and TPDM extract of *Tridex procumbens* Linn. (100 mg/kg p.o. Body weight) respectively. Fourth group received Chlorpheniramine maleate (10 mg/kg, i.p.) was used as standard. All the groups were received clonidine (1 mg/kg s.c.) one hour after the drug administration and the duration of catalepsy was measured at 15, 60, 120 min.

3. STATISTICAL DATA ANALYSIS:

Results were presented as mean ± SEM and analyzed using one way analysis of variance (ANOVA) followed by Dunnett's test for individual comparison of groups with control. The value of P <0.05 was considered statistically significant.

4. RESULTS:

4.1 phytochemical analysis:

Fractions of DFDM & TPDM extracts of were screened for phytochemical analysis table 1 shows the presence of phytoconstituents may be responsible for antioxidant, anticancer and antiparkinson's activity.

Table 1: Preliminary photochemical analysis of DFDM-I & TPDM-IFractions

Sr.No	Phytochemical test	Test applied	DFDM-I Fraction	TPDM-I Fraction
1	Alkaloids	Dragendroff's Test	+++	++
2	Flavonoids	Lead acetate Test	++	+++
3	Glycosides	Modified Borntrager's Test	+++	++
4	Phenolic compound	Ferric Chloride Test	+++	+++
5	Tannins	Gelatine Test	++	+++
6	Saponins glycosides	Froth Test	++	++
7	Phytosterols-	Salkowski Test	+++	++
8	Steroids	Liebermann- Burchard reaction	+++	+++
9	Diterpenes	Copper acetate Test	-	++

*(- = negative; + = slightly positive; ++ = moderate positive; +++ = highly positive)

4.2. Quantitative separation of Rutin by HPTLC analysis:

HPTLC Chromatogram analysis for std. Rutin, DFDM-I& TPDM-Ifractions measured at 366 nm **Fig.1- Fig.3, Table 2.Fig.4- Fig.5, Table 3, Fig.6**

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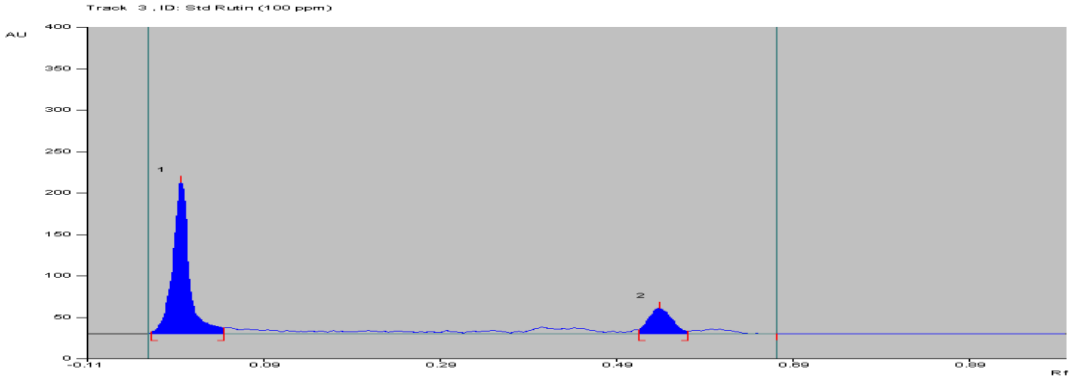
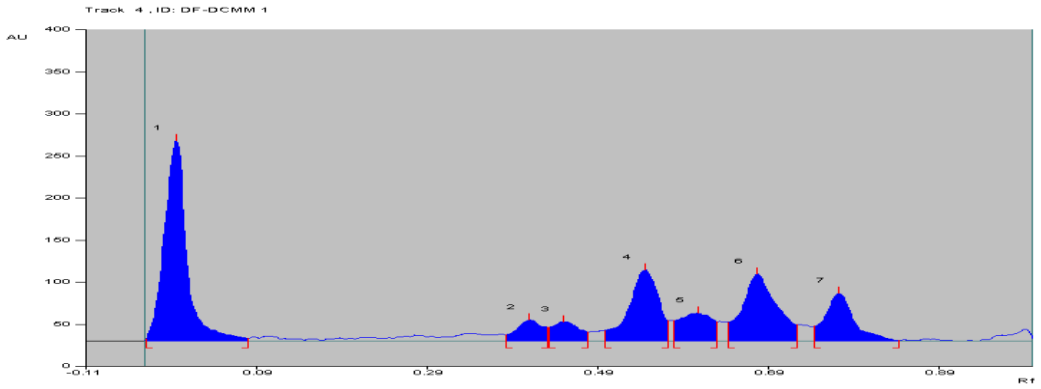
Chromatogram-1

Fig. 1: HPTLC Chromatogram for std. Rutin (1µl)



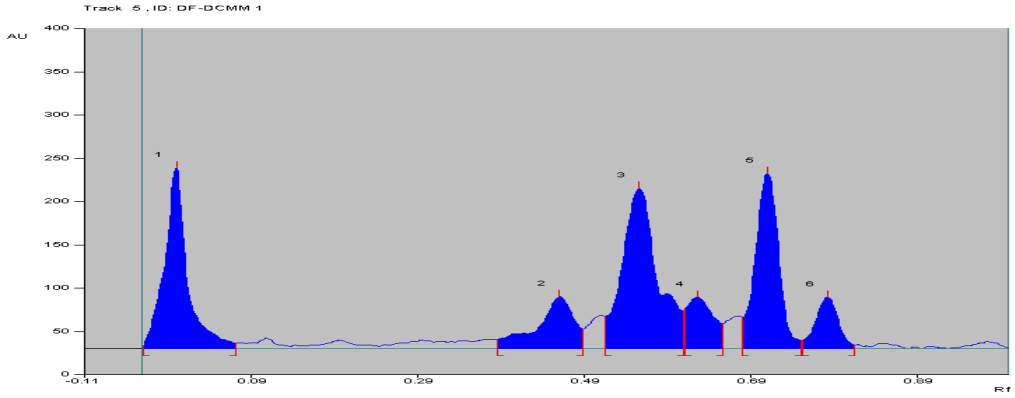
Chromatogram-2

Fig. 2: HPTLC Chromatogram for DFDM-I fraction (1µl)



Chromatogram-3

Fig. 3: HPTLC Chromatogram of DFDM-I fraction (5µl)



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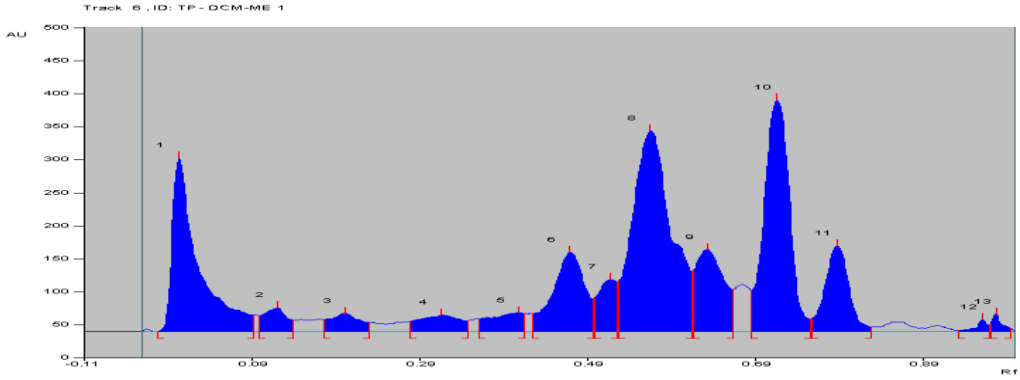
Chromatogram-4

Fig. 4: HPTLC Chromatogram of TPDM-I fraction (1µl)



Chromatogram-5

Fig. 5: HPTLC Chromatogram of TPDM-I fraction (5µl)

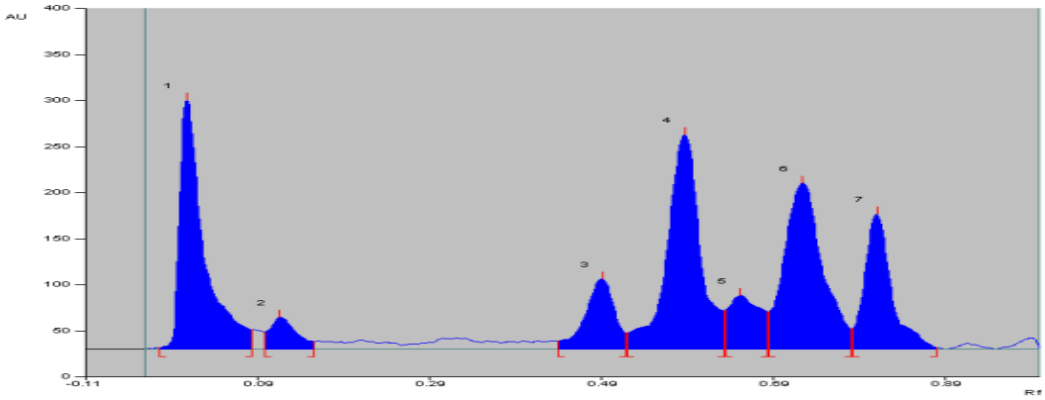
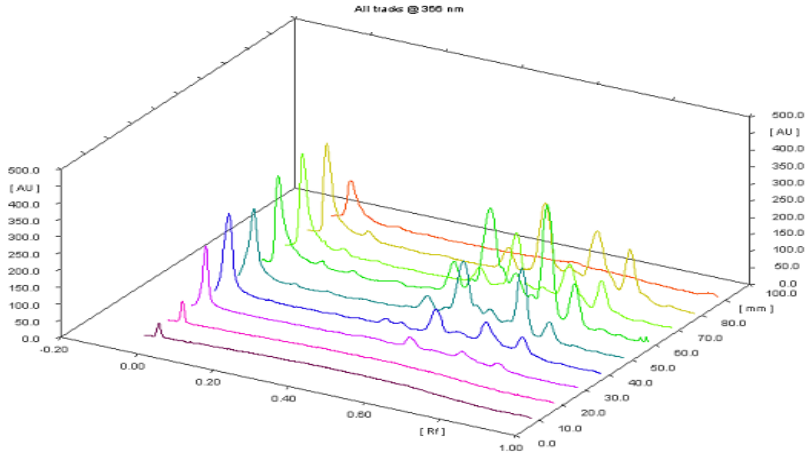


Fig. 6:3D Graph for quantitative separation of Rutin by HPTLC *from* DFDM- I and TPDM-I fraction at



366 nm

Table 2: Quantitative separation of Rutin by HPTLC from DFDM-Ifraction

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Compound/ Fraction	**Concentration of rutin** (measured at 366 nm)			
	Concentration	**Peak No.**	**Rf value**	**Area**
Std Rutin	1µl	4	0.54	2418.6AU
DFDM-Ifraction.	1µl	4	0.54	2418.6AU
	5µl	4	0.55	6580.6AU

Table 3: Quantitative separation of Rutin by HPTLC *from* of TPDM I fraction

Compound/ Fraction	**Concentration of rutin (**measured at **366 nm)**			
	Concentration	**Peak No.**	**Rf value**	**Area**
Std Rutin	1µl	4	0.54	2418.6AU
TPDM-**Ifraction**	1µl	8	0.57	5640.1AU
	5µl	4	0.57	7469.3AU

4.3 GC-MS analysis:

Compounds eluted and identified in DFDM-I fraction by GC-MS analysis are Rhodoxanthin ($C_{40}H_{50}O_2$) Rt.- 25.4 min, Mol.wt.562, Astaxanthin($C_{40}H_{52}O_4$) Rt.- 31.0 min, Mol.wt.596, Squalene ($C_{30}H_{50}$), Rt.- 20.3 min, Mol.wt.410 and fromTPDM-I fractionSqualene ($C_{30}H_{50}$), Rt.- 22.081min, Mol.wt.410. **Fig.7 – Fig-13**

Fig. 7: GC Chromatogram for DFDM-I fraction

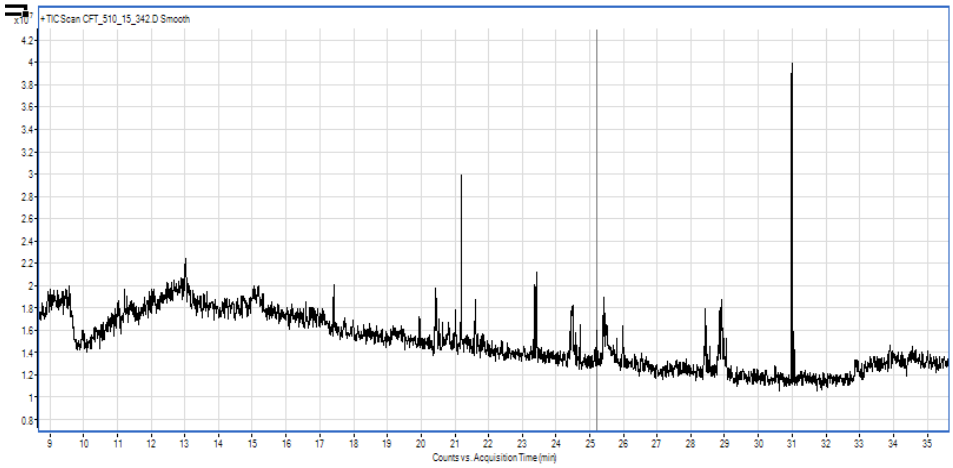

Fig.8: GC-MS spectrum of Rhodoxanthin

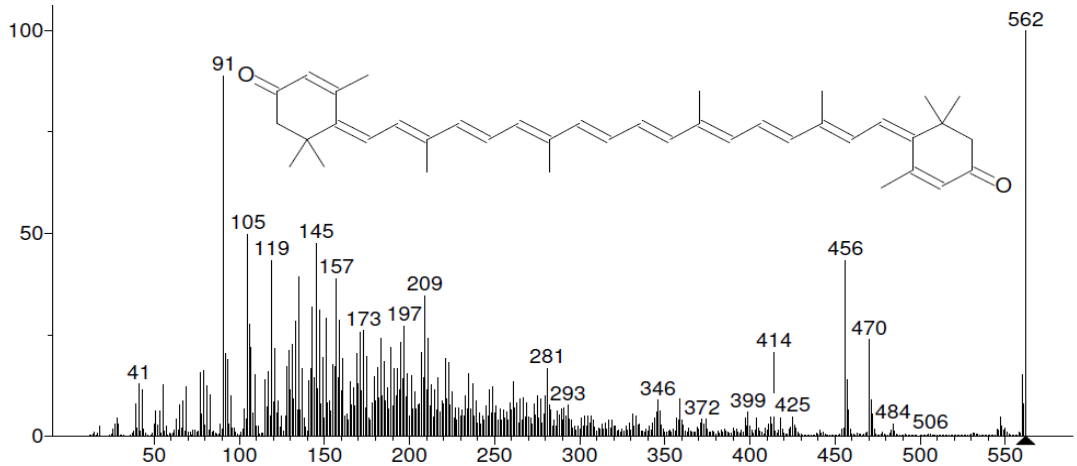

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Fig. 9: GC-MS spectrum of Astaxanthin

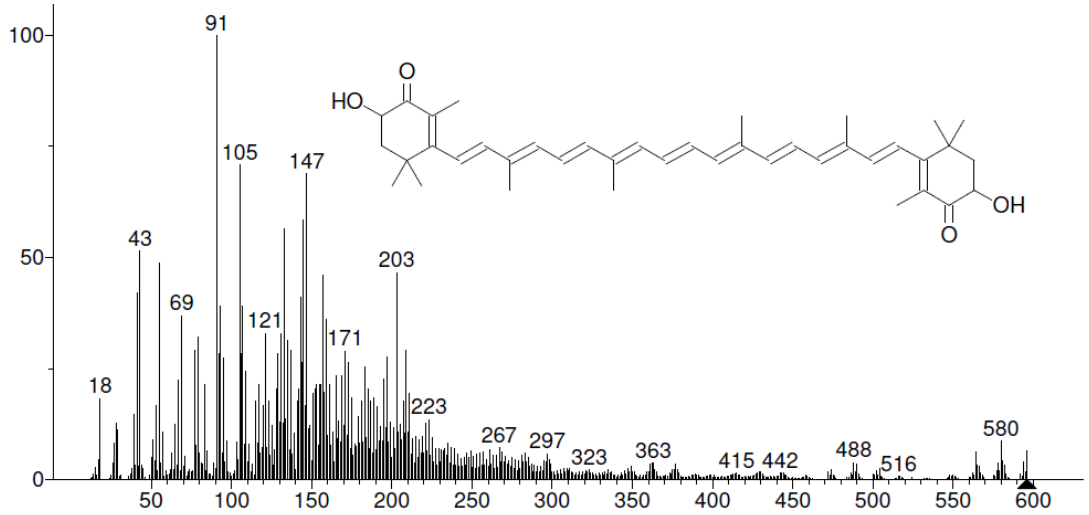

Fig.10. GC: Chromatogram for DFDM –Ia fraction

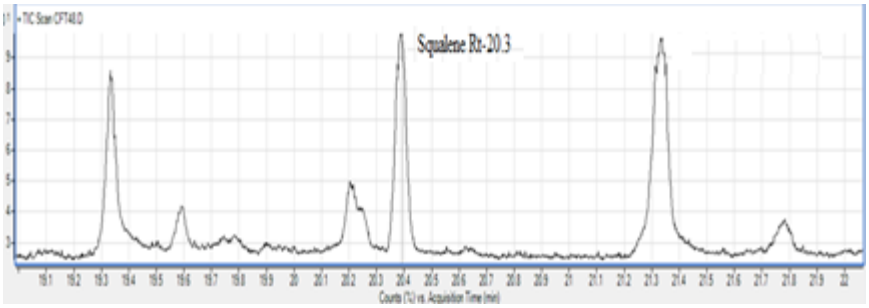

Fig. 11: GC Chromatogram forTPDM-I fraction

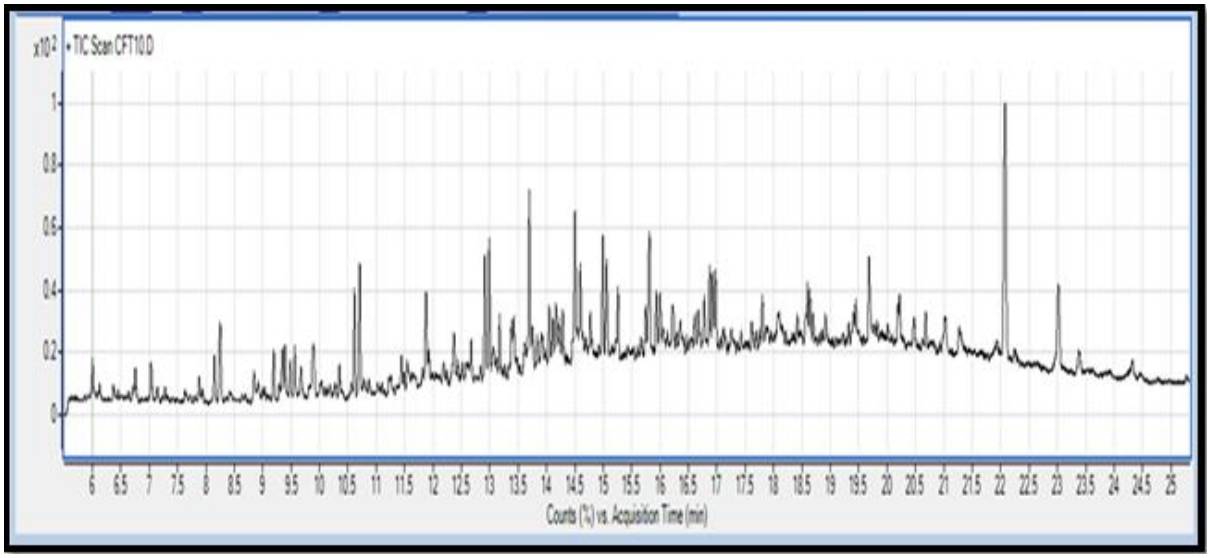

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Fig. 12: GC Chromatogram for TPDM-1aFraction

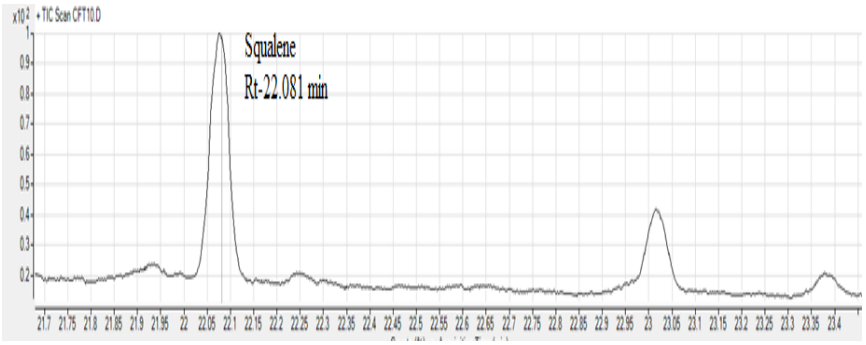
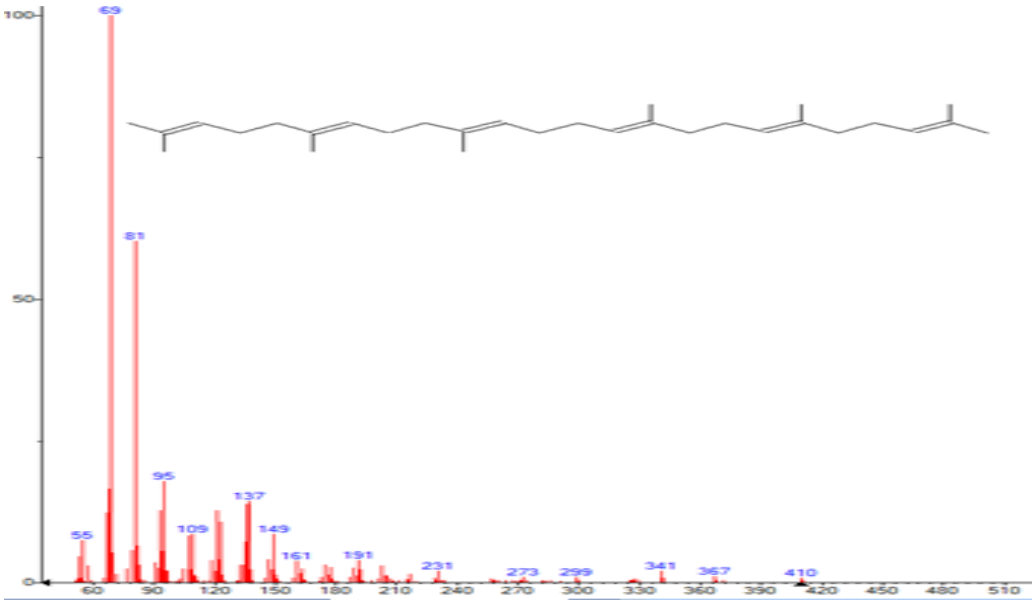


Fig. 13: GC-MS spectrum of Squalene



4.4. Antioxidant activity:

4.4.1. Determination of total phenolic content:

The total phenolic content for DFDM and TPDM extract of *Dendrophthoe falcata* (leaf) and *Tridax procumbens* Linn were estimated by Folin Ciocalteu's reagent method using gallic acid as standard. The dark blue colour produced due oxidation of phenols, having maximum absorbance measured at 760 nm and it directly proportional to total phenol compound present. The gallic acid solution conc. 1- 10 µg/ml it obeys the beer's law with regression co-efficient (R^2 = 0.9964) .The plot has lope (m)=0.01 and intercepts = 0.0029. Std. Equation of curve is Y= 0.01 x +0.0029.

Table 4: Total phenolic content for extracts

Sr.No.	Extracts Concentration	Absorbance at 760 nm	Total Phenolic content (mg of gallic acid equivalent/ g dry wt.)
1	DFDM (20 mg/ml)	3.429	17.44
2	TPDM (20 mg/ml)	1.676	8.524

4.4.2. Reducing power assay:

Reducing power assay measurement is base on absorbance of ascorbic acid and Absorbance of ascorbic acid equal to be 100% antioxidant activity. Maximum absorbance of extracts and ascorbic

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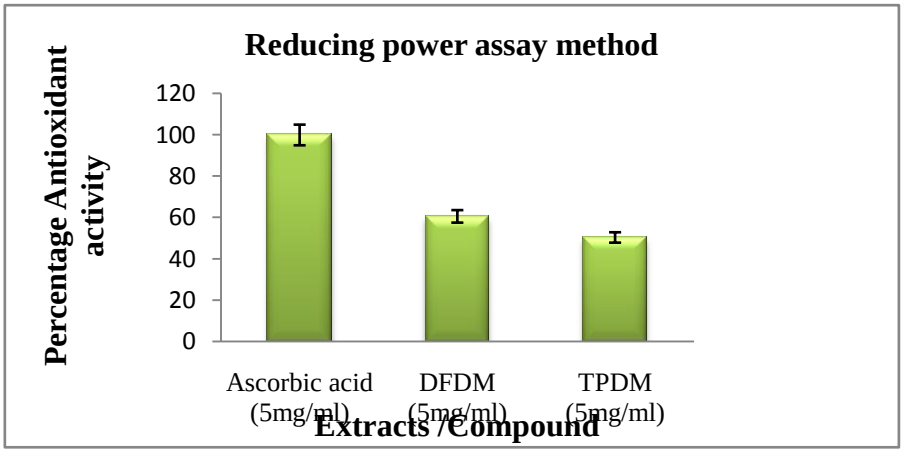
acid combination is equal to the maximum reducing power. Results show that the antioxidant activity of DFDM & TPDM extracts. The reducing power of the DFDM, TPDM were found to be 60.62%, 52.66% respectively. **Fig.14**

Fig. 14: Antioxidant activities (reducing power assay) of extracts of DFDM and TPDM



4.5. ANTICANCER ACTIVITY:

4.5.1. MTT assay:

The cytotoxicity study of DFDM, TPDM extracts and Cisplatin (positive control) evaluated against Cell lines. DFDM extracts having IC_{50} value for A549 (20 µg/ml), HEK-293(< 20µg/ml), Hela (<30µg/ml), K-562(20µg/ml), Cisplatin (10 µg/ml) and TPDM extracts IC_{50} value forA549 (10 µg/ml), HEK-293(>30µg/ml), Hela (<30µg/ml), K-562(< 20µg/ml), Cisplatin (10 µg/ml)by MTT assay. **Fig.15-Fig.16**

Table 5: Cytotoxicity of DFDM and TPDM extracts against Human kidney Cell line-HEK-293 by MTT assay

Extracts/ Compounds	**Concentration (µg/ml)**	**O.D. at 490 nm**	**%Cell viability**	**% cell lysis**	**IC_{50} µg**
DFDM	10	1.556±0.016	58.28±1.721	41.72±1.721	< 20µg/ml
	20	1.236±0.006	47.04±1.863	52.96±1.863	
	40	0.936±0.0028	35.06±2.312	64.94±2.312	
	80	0.567±0.0055	21.27±1.875	78.72±1.875	
TPDM	10	2.797±0.0038	100	00	>40µg/ml
	20	2.342±0.0561	87.71±3.050	12.29±3.050	
	40	1.620±0.0012	60.67±5.558	39.33±5.558	
	80	0.993±0.0151	37.19±3.65	62.81±3.65	
Cisplatin	10	0.368±0.0038	13.78±1.56	86.21±1.56	< 10µg/ml
Control	00	2.670±0.001	100	00	-

Fig. 15: % Cell viability (Cytotoxicity) of DFDM extract against HEK-293, cell line by MTT assay

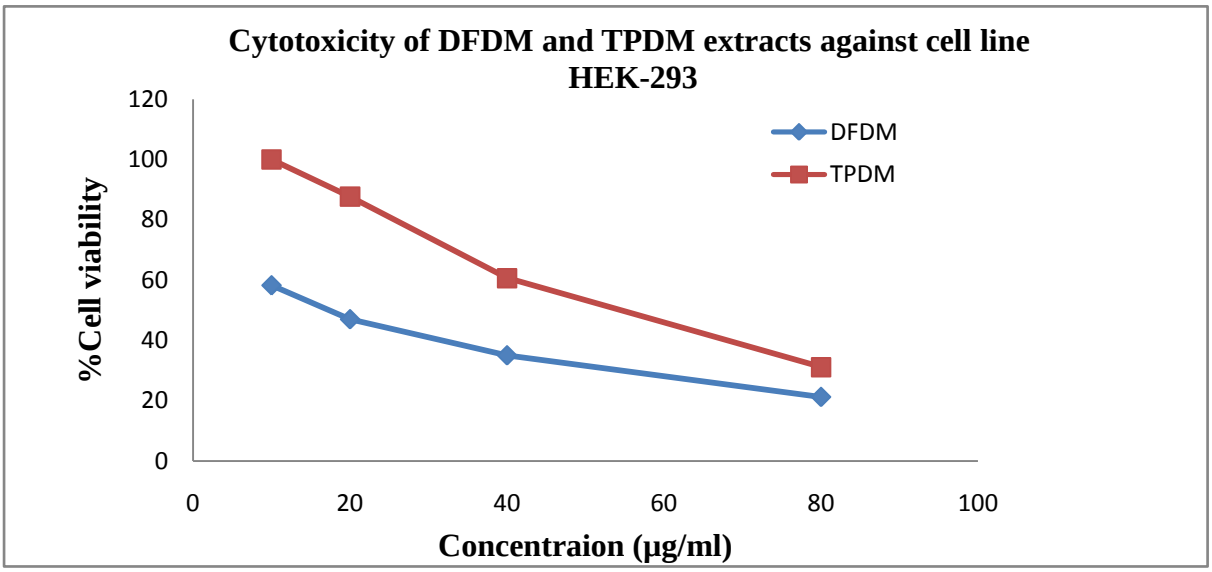


Table 6: Cytotoxicity of DFDM and TPDMextracts against Human cervix Cell line - Helaby MTT assay

Extracts	**Concentration (µg/ml)**	**O.D. at 490 nm**	**%Cell viability**	**% cell lysis**	**IC_{50} µg/ml**
DFDM	10	1.849±0.0056	82.81±1.871	17.19±1.871	< 40µg/ml
	20	1.587±0.0095	71.08±0.778	28.92±0.778	
	40	0.939±0.0088	42.06±1.128	57.94 ±1.12	
	80	0.762±0.009	34.16±1.635	65.83±1.635	
TPDM	10	1.721±0.019	77.10±0.851	22.9±0.851	>20µg/ml
	20	1.177±0.070	52.73±1.171	47.27±1.171	
	40	0.790±0.010	35.41±0.523	64.59±0.523	
	80	0.464±0.032	20.80±1.746	79.20±1.746	
Cisplatin	10	0.286±0.019	12.80±1.46	87.19±1.46	< 10µg/ml
Control	00	2.233±0.001	100	00	-

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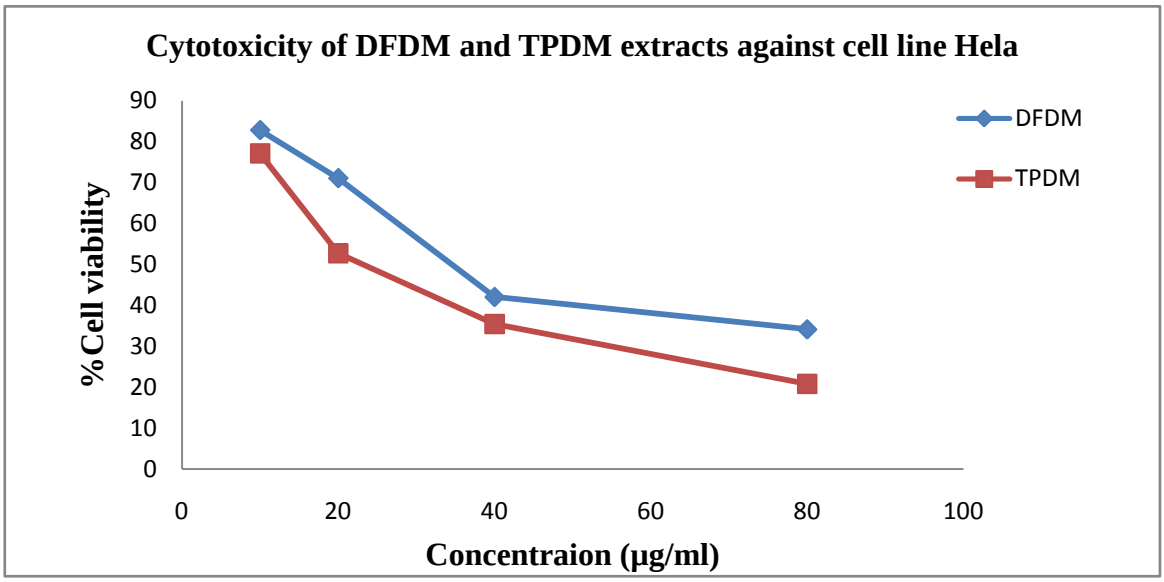
Fig.16: % Cell viability (Cytotoxicity) of TPDM extract against HEK-293, HELA, cell line by MTT assay



4.6. ANTI-PARKINSON ACTIVITY:

4.6.1. Haloperidol induced Catalepsy activity:

In catalepsy bar test the group which received haloperidol, significant increase in latency period ($p<0.001$) was seen on day "0" and the day "07" as compared to the control group. In levodopa treated group, a significant decrease in the latency period ($p<0.001$) on day "0" and the day "07" was seen as compared to haloperidol treated group. However, on day "07" DFDM extract (100 mg/kg) groups showed significant decrease in latency period ($p<0.01$) when compared to haloperidol treated group as shown in **Table 7**,whereas, no significant difference in latency period was seen when TPDM extract (100 mg/kg) treated group compared to levodopa treated group.**Fig.17**

Table 7: Effect ofDFDM and TPDM extracts on catatonic response in haloperidol treated mice

Sr. no.	**Groups**	**Dose**	**Latency period (sec) 0 day**	**Latency period (sec) 7th day**
1	Control vehicle	1 ml/ kg PO	13.04 ± 0.50	24.08 ± 1.46
2	Haloperidol (Hal.)	1 ml/kg I.P.	121.8 ± 2.76	130.8 ± 1.06
3	Levodopa	30mg/kg i.p + Hal.	87.02 ± 1.15**	64.00 ± 1.41**
4	DFDM Extracts	100 mg/kg, p.o. + Hal.	146.60 ± 1.53**	88.06 ± 1.43**
5	TPDM Extracts	100 mg/kg, p.o. + Hal.	149.80 ± 1.53**	131.4 ± 1.36**

n=06, values expressed as mean± SEM.*p<0.05, **p<0.01, compared to control group

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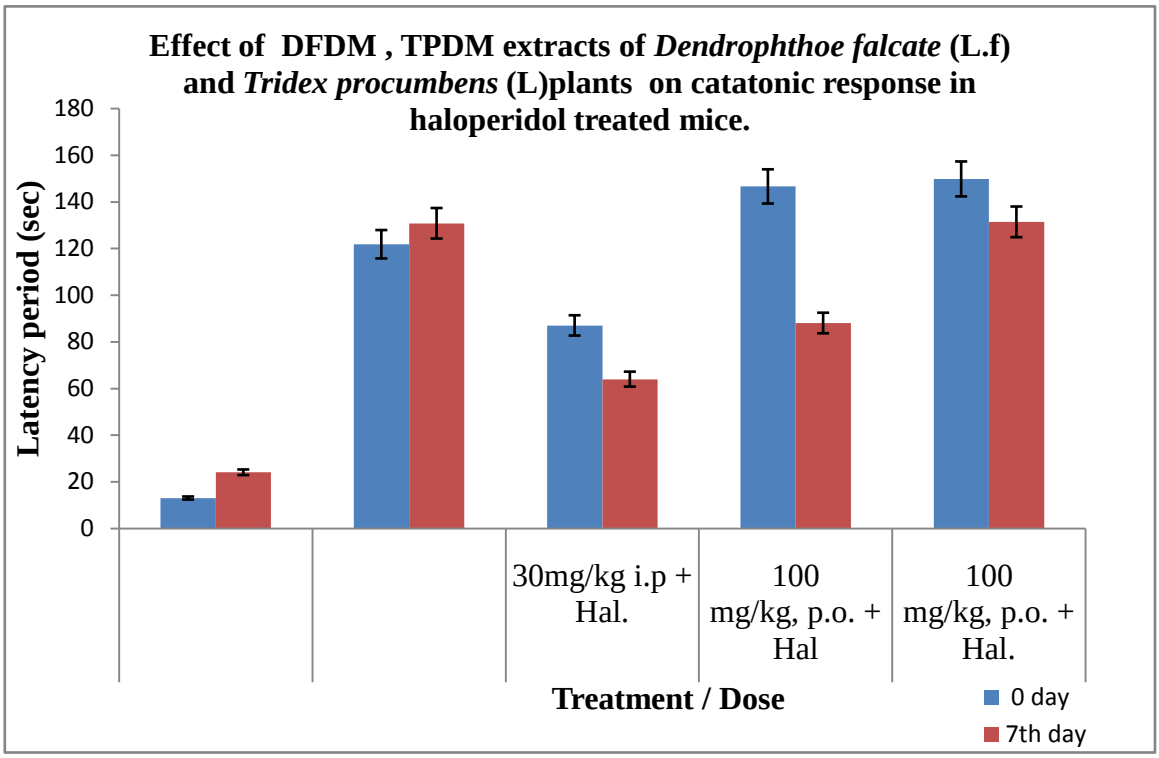
Fig.17: Effect of DFDM and TPDM extracts *on* catatonic response in haloperidol treated mice



4.6.2 Clonidine induced catalepsy study in mice:

Clonidine (1mg/kg, S.C.) produced catalepsy in mice, which remained for 2 hours. The vehicle treated group has shown maximum duration of catalepsy (120 ± 3.5sec.) at 120 minute after the administration of clonidine. There was significant inhibition ($p<0.05$, $p<0.01$) of Clonidine induced catalepsy in the animal pre-treated with DFDM extracts of *Dendrophthoe falcate* (L.F) and TPDM extracts of *Tridex procumbens* Linn (100 mg/kg, p.o.) and the duration of catalepsy was found to be 59.60 ± 1.50, 54.80 ± 1.15 seconds respectively. Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed ($p<0.01$) the Clonidine induced catalepsy in mice. (**Table 8) Fig.18.**

Table 8: Effect of DFDM) and TPDM extract on clonidine induced catalepsy in mice

Group	**Treatment**	**Duration of catalepsy (sec) at Mean ±SEM**		
		15 min	**60 min**	**120 min**
Group-I	Vehicle (10ml/kg, i.p.)	62.80 ± 1.46	75.00 ± 2.38	83.60 ± 1.12
Group-II	Chlorpheniramine maleate (10 mg/kg, i.p.)	23.80 ± 0.86**	20.60 ± 0.92**	25.60 ± 1.32**
Group-III	DFDM extract (100 mg/kg, p.o.)	42.60 ± 0.92**	59.60 ± 1.50**	66.00 ± 0.70**
Group-IV	TPDM extract (100 mg/kg, p.o.)	36.60 ± 0.92**	54.80 ± 1.15**	60.20 ± 1.15**

n=06, values expressed as mean± SEM. *p<0.05, **p<0.01, compared to control group

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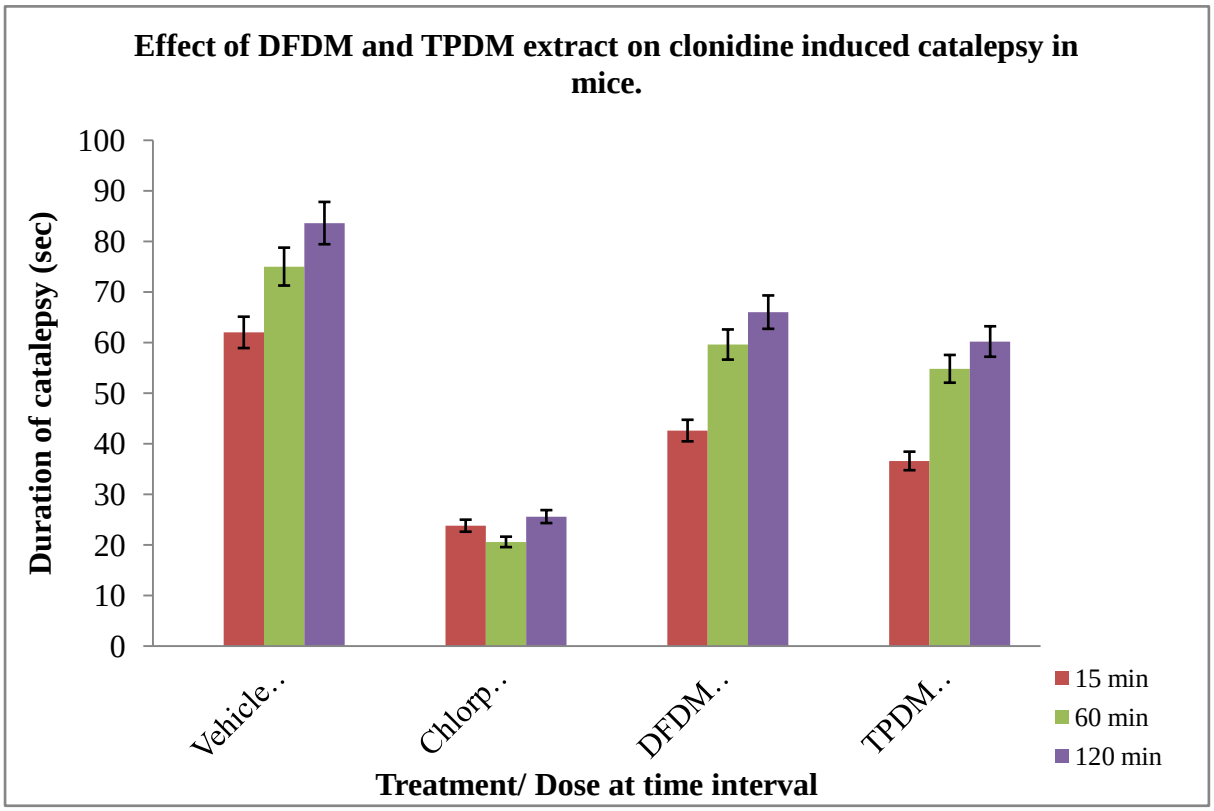
Fig. 18: Effect of DFDM and TPDM extracts on clonidine induced catalepsy in mice



5. DISCUSSION:

Preliminary phytochemical screening offractions extracts DF and TP plant shows presences of Secondary metabolite alkaloids, flavonoids, saponins, tannins, phenolic group, glycosides,phytosterols and terpenoids responsible for pharmacological activity. Quantitative HPTLC study fractions of DFDM and TPDM (1µl/ml & 5µl/ml) extracts show presence of rutin at an R_f value 0.54 and 0.57 having Area 2418.6AU & 6580.6AU and 5640.1AU 7469.3AU compare std rutin R_f value 0.54 (1µl/ml) having Area 2418.6AU. Separation based concentration also reflected in 3D graph of HPTLC chromatogram. (**Fig. 6**.) Literature also revels the presence of Rutin as polyphenolics phytoconstituents in *Dendrophthoe falcate* (L.f) and *Tridax procumbens* (Linn). Rutin act as potent antioxidant, anticancer, neuroprotective, anticonvulsant, antimicrobial, and hepatoprotective phytomoiety.

Rutin act by protecting dopaminergic neurons from oxidative stress as a potent antioxidant in Parkinson deceases (PD). [42] Parkinson's disorder is neurodegenerative disease due to loss of dopamine neurons in substantia nigra, synaptic vesical. Dopamine level decreases due to oxidative stress, neuronal cell toxicity. Oxidative stress responsible for PD. Reactive oxygen species regenerates more dopamine in substantia nigra and rutin work through ROS, free radical scavenging activity (antioxidant), Rutin reduces hydrogen peroxide-induced oxidative stress in epithelial cells [43-44].Result and literature show that rutin repair the PD by removal of oxidative stress as strong ROS.

GC-MS analysis of DFDM extracts *Dendrophthoe falcate (L.f)* plant shows presence of

Rhodoxanthin and astaxanthin are naturally functioning carotenoids. Rhodoxanthin is good antioxidant act by inhibiting cell proliferation in cancer cell lines. Rhodoxanthin act by arresting cell in its S-Phase of cell cycles and decrease mitochondria transmembrane potential and level of intracellular Ca^{2+} increase and through this mechanism

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rhodoxanthin act as anticancer agent [45]. Astaxanthin show the antioxidant activity via peroxyl radical scavenging among all carotenoids

GC-MS analysis of fraction of DFDM extracts identified Squalene –Rt-20.392 min, (**Fig-10**) Squalene is antioxidant triterpene act by oxygen scavenging activities. Squalene strongly act antioxidant by withdraw or donate electron from molecule. Squalene is good anti tumour agent act via strong inhibitory activity of HMG-COA reductase catalytic activity. [46]

Within study results shows that antioxidant activity was due to phenolic compounds. Literature show that reducing power of compounds is may be due to their hydrogen donating capability. Reducing power assay used to study ability of antioxidant to donate electrons and that is the mechanism of action of phenolic compound as an antioxidant. The study show that extracts of *dendrophthoe falcate* (L.f)*& tridax procumbens* (Linn)contains polyphenols which may responsible for antioxidant activity.[47]

Antioxidant potential of phenols is mostly due to their redox behaviour, singlet oxygen quenchers and hydrogen donors.[48] The ratio of antioxidant activity of extracts is directly proportional to total phenolic contents of plant extracts.[49]Reducing power assay within recent study shows that DFDM & TPDM extract of *dendrophthoe falcate* (L.f)*& tridax procumbens* (Linn) maximum amount of the total polyphenols. Antioxidant property of plant extracts useful to prevent neurodegenerative disorders.

The cytotoxicity study of DFDM & TPDM extracts evaluatedagainstHEK-293, HeLa cell lines Cell line by MTT assay. IC_{50} value calculated compare to control, positive control & DFDM extracts show potent activity against Cell line-.HEK-293 TPDM extracts show potent activity against Cell line HeLa cell. DFDM extract shows presence of Rutin (**Table 2 & 3**), Rutin inhibit cancer cell growth by cell cycle capture or by apoptosis, with inhibition of proliferation, metastasis of cell via antioxidant activity. Free radical scavenging activity, superoxide anion generation was key factor for cell lysis. Rutin stops the growth of cancer cell via decreases the ROS generation and reduces oxidative stress, DNA damage due to its antioxidant property. Rutin also incorporated into cell membrane and block cell cycle development in to GO/G1 phase, G2/M-phase, S-phase and reduces cancer cell production [52].Rhodoxanthin and other flavonoids, phytosterolsmay be responsible for activity. DFDM extract shows presence of phytosterols has ability to incorporate to cell and block cell cycle development. [50-51] Cytotoxicity due to presence of Squalene and other phytosterols, sesquiterpenes in crude and fractions.

In catalepsy bar test the group which received haloperidol, significant increase in latency period ($p<0.001$) was seen on day "0" and the day "07" as compared to the control group. The extracts of DFDM and TPDM extracts exhibited a dose dependent and significant ($P < 0.01$) reduction catatonic responsehaloperidol induced Catalepsy activity. Catatonic response of negative control 130.8 ± 1.06 reduces to 88.06 ± 1.43 by DFDM extract (100 mg/kg) significant than TPDM extract (100 mg/kg) 131.4 ± 1.36 reference to levodopa (30mg/kg) 64.00 ± 1.41**.** Significant inhibition in clonidine induce catalepsy in mice ($p<0.05$, $p<0.01$) in the animal pre-treated with DFDM extracts and TPDM (100 mg/kg, p.o.) and the duration of catalepsy was found to be 59.60 ± 1.50, 54.80 ± 1.15 seconds respectively.

The results of separation and Pharmacological study of crude and fraction provide the basis for mechanism of action of phytochemical. Antioxidant activity of crude and presence of phytochemical combine design the action as anticancer, antiparkinson's activity which is significantly proved by the in vitro, in vivo studies. In the fractions DFDM & TPDM

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extractspresence of Rutin, Squalene, flavonoid phytosterols, sesquiterpenes, play major role in activity. Anti Parkinson activity of DFDM & TPDM extracts first time reported in study with significant values with respect to traditional medicinal use of the plant. Cytotoxicity study of DFDM and TPDM against HEK-293, HeLa cell lines provide best data for development of new phytochemical anticancer medicine which is socially benefit in future.

CONFLICT OF INTEREST STATEMENT: The authors declare no conflict of interest.

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