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Original Research Article

QUALITATIVE ASSESSMENT OF ANTIOXIDANT POTENTIAL IN VARIOUS DOSAGE FORMS OF *Terminalia arjuna*

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ABSTRACT:
Medicinal Plants, as a source of remedies, are widely used as alternative therapeutic tool for the prevention or treatment of many diseases. Our main objective was to evaluate the antioxidant activities of solvent extracts from *Terminalia arjuna*, and to choose a dosage form with promising activity for further studies. The antioxidant activity was assayed by DPPH (Free radical scavenging activity) and Lipid peroxidation method.

KEYWORDS : *Terminalia arjuna*, Anti-oxidant potential

INTRODUCTION

Arjuna consists of dried stem bark of *Terminalia arjuna*, found as naturally growing plant in dense forests.

SCIENTIFIC CLASSIFICATION	
Kingdom	**Plantae**
Division	**Magnoliophyta**
Class	**Magnoliopsida**
Order	**Myrtales**
Family	**Combretaceae**
Genus	**Terminalia**
Species	***T.arjuna***

The objective of study is to evaluate the anti-oxidant activities of various dosage forms of *Terminalia arjuna*, by employing DPPH radical scavenging activity method and Lipid peroxidation method.

The thick, white-to-pinkish-grey bark has been used in India's native Ayurveda for over three centuries, primarily as a cardiac tonic. Clinical evaluation of this botanical medicine indicates it can be of benefit in the treatment of coronary artery diseases, heart failure and possibly hypercholesterolemia. It has also been found to be antiviral and anti-mutagenic. Terminalia's active constituents include tannins, cardenolide, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc and copper.[1-4]

MATERIALS AND METHODS

Reagents used

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Chloroform (HPLC Grade), p^H 7.4 Tris buffer were used for the analysis of drug. Chloroform (HPLC grade) was procured from Loba chemicals, Hyderabad. Water (HPLC grade) was obtained from a Millipore water purification system. Reference standard was provided from National Institute of Nutrition, Tarnaka, Hyderabad.

Preparation of P^H 7.4 Tris Buffer :

1.211 gm. of Tris was accurately weighed and transferred into a 100ml volumetric flask. To this, 60ml of water (HPLC grade) was added to dissolve Tris and p^H was adjusted to 7.4 with the addition of Hydrochloric acid, and volume is made to 100ml with water.

Preparation of Mobile Phase:

Chloroform (50ml), Ethyl acetate (40ml) were mixed and degassed it with sonicator for 10 minutes. The resultant solution was used as mobile phase.

Preparation of diluent :

10 mg of *Terminalia arjuna* was accurately weighed portion of the powder (10.012mg), equivalent to 10 mg of *Terminalia arjuna* was transferred into a 10 ml volumetric flask 4ml of diluent was added, sonicated for 10 min. The volume was made up to mark with diluent and then filtered. 1 ml of the above filtrate was collected, transferred into a 10ml volumetric flask, diluted to volume with diluent.

PROCEDURE FOR ANTI-OXIDANT ACTIVITY: [7]

Anti-oxidant activity study of the drug substance and the drug product were performed by DPPH Assay and induction and assay of Lipid Peroxidation.

DPPH ASSAY (Free Radical Scavenging Activity measured by 1,1-Diphenyl-2-picryl-hydrazil) [15,16]

1. DPPH: 0.15mM of DPPH is prepared by dissolvin g 2.95mg DPPH in 50 ml of absolute alcohol. (NOTE: DPPH solution dhould be made freshly and should be kept in dark).
2. TRIS BUFFER: Dissolve 1.21gm of Tris Hcl in distilled water about 75ml and adjust p^H 7.4 with dilute HCL followed by makeup the volume to 100ml with distilled water.
3. The potential antioxidant activity of dosage forms has been determined in comparison with the standard preparation viz, ascorbic acid by calculating 50% inhibitory concentration (IC50) values.
4. Stock Solution: 1mg/ml stock solution is prepared by dissolving 10mg of ascorbic acid in 10ml Millipore water and make up volume in a standard flask. Working standard solution is prepared from the stock solution by 0.1ml of stock solution and making up the volume to 10ml with Millipore water to prepare 10µg/ml solution.
5. Aliquot samples are taken from the working standard to plot the standard graph ranging from the 10µg to 20µg solutions.
6. The volume is made to 1ml with Tris HCL buffer.
7. 1ml of the 0.15mM alcoholic DPPH solution was added in the above test tube.
8. The above solutions are wrapped with the dark paper to avoid he effect of light and allow the solutions to stand for 20min in the dark.
9. After 20 min absorbance of the solution is measured at 517nm. [The triplicate readings of each dilution is taken to measure the optical density (O.D)] Optical density is taken by the subtraction of O.D of blank from sample.
10. Interpretation: The graph is plot between the O.D on y-axis and concentration on x-axis. Using equation $y = mx + C$ O.D at 1µg and

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10µg is taken to make a graph. IC50 value of the vitamin-C is determined by taking the O.D of the buffer and DPPH solution as the 100% absorbance and calculating the percentage of the scavenging activity of Vitamin-C at different concentration by subtraction from the 100% absorbance to find out the percentage of the scavenging activity.

11. Calculation

$$IC50 = \frac{\text{O.D of Control} - \text{OD of Test}}{\text{O.D Control}} \times 100$$

12. Where OD control is the initial absorbance and OD sample the value for added sample concentration. The quantity of test compound has been selected to obtain the linear concentration (Beer Lambert's law) grade dose information.

DPPH Scavenging Effect

(50%) = $[(A_0 - A_1)x100]$

Where,

A_0 was the absorbance of the control reaction

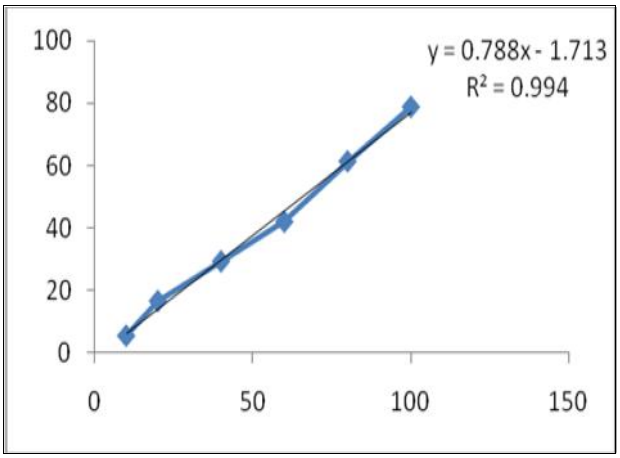
A_1 was the absorbance in the presence of the sample (Arjuna Brands).

S.No	PRODUCT	DPPH ASSAY (IC-50)
1	TABLET	10.60
2	CAPSULE	61.60
3	POWDER-1	14.86
4	POWDER-2	15.86
5	CHOORNAM	77.84
6	BARK	15.96

The Percentage inhibition exhibited by Vitamin-c is taken as a standard reference and the scavenging effect of the Arjuna products is calculated.

VITAMIN – C

Concentration	%Inhibition	
1	5.60	
2	16.71	
4	29.44	IC 50 6.55
6	42.13	
8	61.46	
10	78.86	



Arjuna Tablet
ARJUNA CHHAL GHANVATI
Ayurvedic Medicine,
Batch no : 104
Mfg date : Apr-2010

Concentration	% Inhibition	
1	4.80	
2	7.50	
4	9.73	IC 50 29.87
6	14.53	
8	17.23	
10	18.15	

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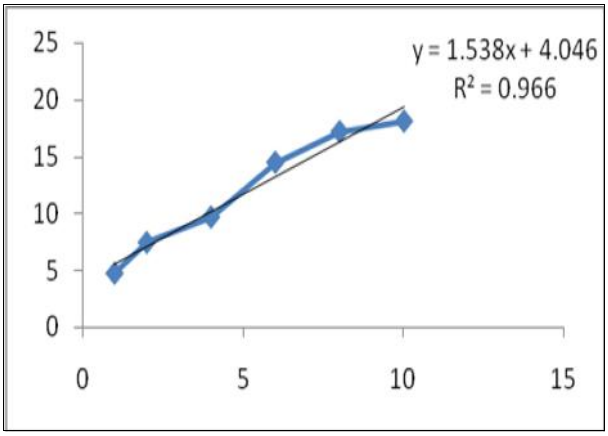
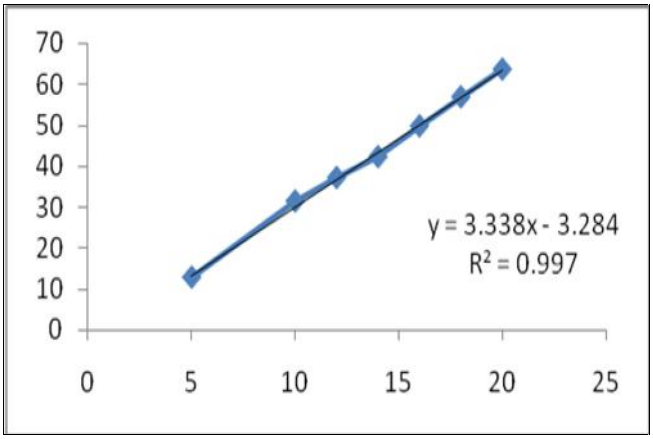


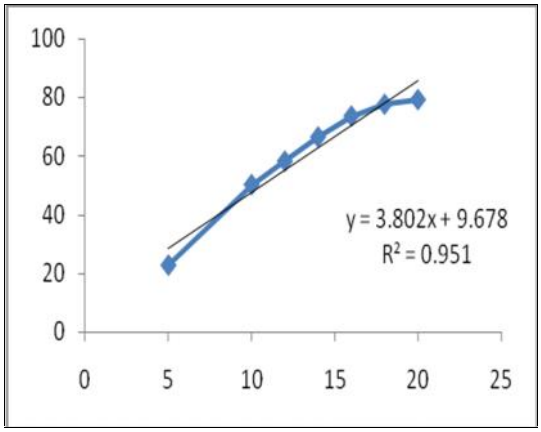
Arjuna Powder-1
Arjuna Powder (50g)
Batch no : 1
Mfg. date: Feb 2011

Concentration	% Inhibition	
5	0.66	
10	0.52	IC 50 10.60
12	0.47	
14	0.43	
16	0.38	
18	0.32	
20	0.27	

Arjuna Capsules
Arjuna
Batch no : F 0210046
Mfg date : June-2011

Concentration	% Inhibition	
5	23.02	
10	50.39	IC 50 10.60
12	58.35	
14	66.57	
16	73.68	
18	77.69	
20	79.27	





Arjuna Powder (Fine)
Batch no : 18
Mfg. date : Dec 2010

Concentration	% Inhibition	
1	8.42	
2	11.05	IC 50 13.60
4	18.02	
6	25.92	
8	26.90	
10	40.65	

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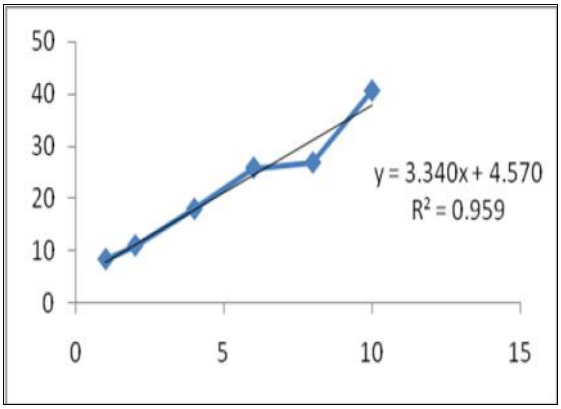
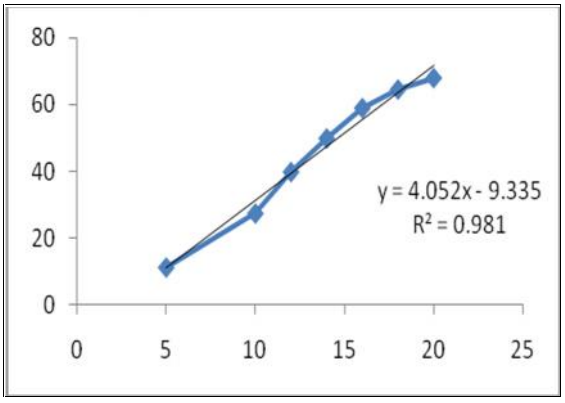


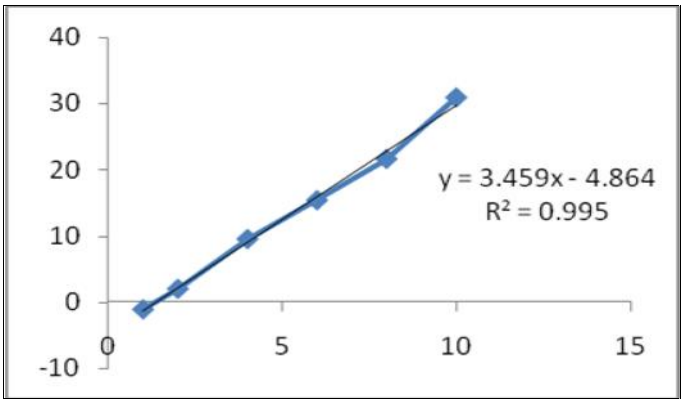
Arjuna Choornam
Arjuna Choornam (100g)
Batch No : 007
Mfg. date : 07/2011

Concentration	% Inhibition	
5	11.18	IC 50 14.64
10	27.36	
12	39.80	
14	49.86	
16	58.94	
18	64.53	
20	67.96	



Arjuna Bark
Obtained from S.K.Dawasaz, Unani & Ayurvedic Chemists Medicinal Herbs
The bark was grinded, powdered and stored.

Concentration	% Inhibition	
1	-1.13	IC 50 15.86
2	1.95	
4	9.47	
6	15.34	
8	21.57	
10	30.84	



INDUCTION AND ASSAY OF LIPID PEROXIDATION[8]

Isolated Rat Mitochondria are used in this assay. Malonaldehyde analysis is an intermediatory by product of this assay.

1. Lipid peroxidation was induced in mitochondria by Fe^{2+}-ascorbate or Fe^{2+}-H_2O_2.
2. Briefly mitochondria (500µg $Feso_4$, 200µM ascorbate and 0.125 M KCl- 0.02 M Tris HCl buffer p^H 7.4) or Fe^{2+}-H_2O_2 system (200µg $Feso_4$, 100µM H_2O_2 and 0.15M

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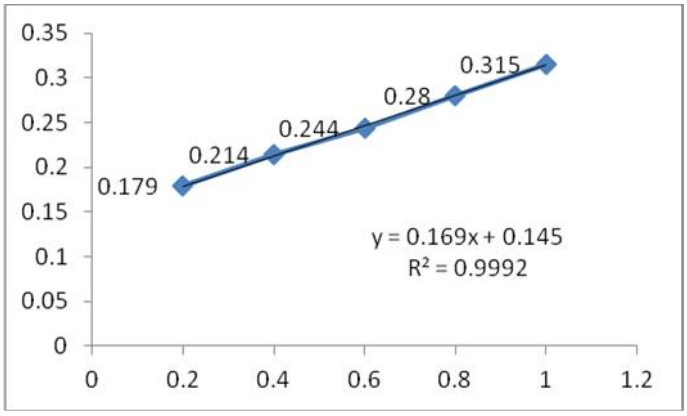
NaCl, p^H 7.0) for a period of 60min at 37°. [15]

3. Inhibitors were added at indicated concentrations 2 minutes prior to addition of peroxide inducers.
4. Peroxidation was terminated by adding 20% Trichloroacetic acid (0.5ml/min incubation mixture).
5. Thiobarbituric acid reactive substances (TBARS) were determined according to Wilbur et al. [14]
6. Acid hydrolysed 1,1,3,3, tetraethoxypropane was used as an authenic standard for MDA.
7. Although it is not specific for MDA, the assay of TBARS is used as a reliable index of lipid peroxidation. [13]
8.

S.no	PRODUCT	LIPID PEROXIDATION % INHIBITION
1	TABLET	40.23
2	CAPSULE	54.62
3	POWDER-1	56.64
4	POWDER-2	82.02
5	CHOORNAM	47.64
6	BARK	44.70

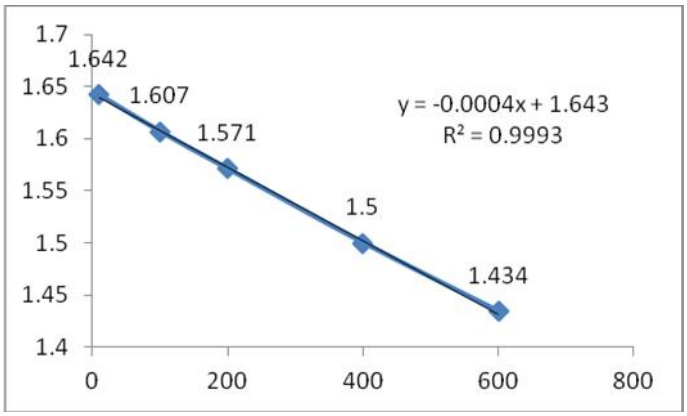
Malonolaldehyde Standard Graph

Concentration (ml)	O.D Mean
0.2	0.179
0.4	0.214
0.6	0.244
0.8	0.280
1	0.315
Blank	0.315



VITAMIN –E

Concentration	O.D	Inhibition Concentration
10	0.38	1.642
100	0.374	1.607
200	0.368	1.571
400	0.356	1.500
600	0.345	1.434



Capsule

Concentration	O.D	Inhibition Concentration
10	0.261	0.934
100	0.254	0.892
200	0.242	0.821
400	0.238	0.797
600	0.222	0.702
800	0.211	0.639
1000	0.198	0.559

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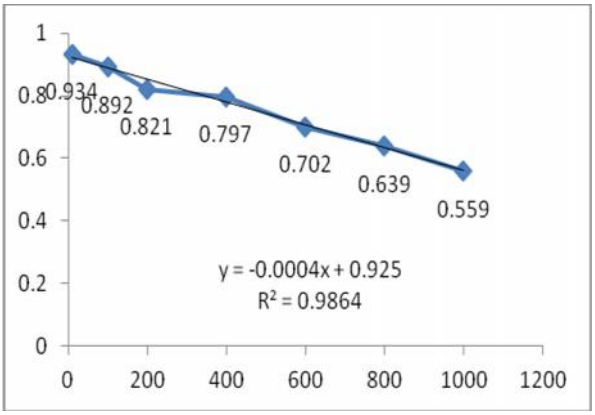
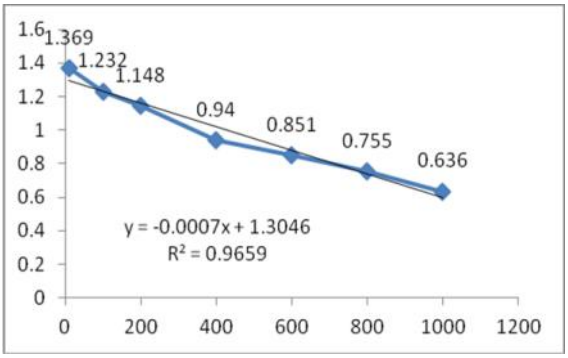


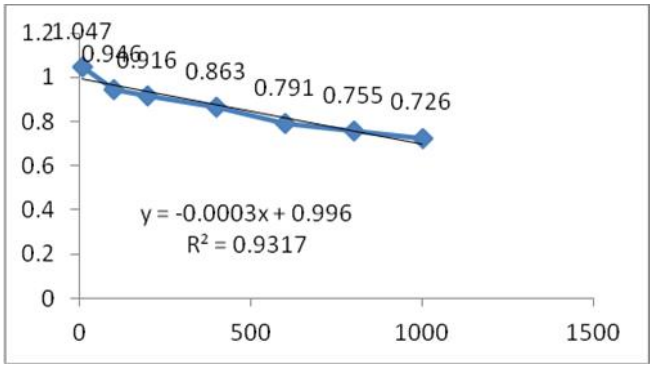
Tablet

Concentration	O.D	Inhibition Concentration
10	0.334	1.369
100	0.311	1.232
200	0.297	1.148
400	0.262	0.940
600	0.247	0.851
800	0.231	0.755
1000	0.211	0.636



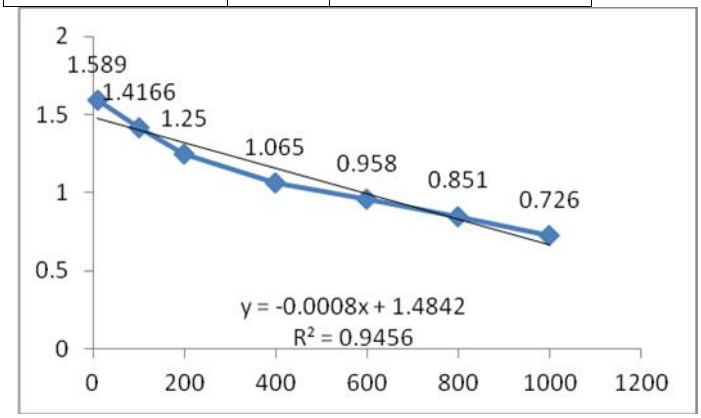
Arjuna Powder-1

Concentration	O.D	Inhibition Concentration
10	0.280	1.047
100	0.263	0.946
200	0.258	0.916
400	0.249	0.863
600	0.237	0.791
800	0.231	0.755
1000	0.226	0.726



Arjuna Powder -2 (Fine)

Concentration	O.D	Inhibition Concentration
10	0.371	1.589
100	0.342	1.416
200	0.314	1.250
400	0.283	1.065
600	0.265	0.958
800	0.247	0.851
1000	0.226	0.726



Choornam

Concentration	O.D	Inhibition Concentration
10	0.231	0.755
100	0.219	0.684
200	0.209	0.625
400	0.197	0.552
600	0.189	0.505
800	0.184	0.476
1000	0.177	0.434

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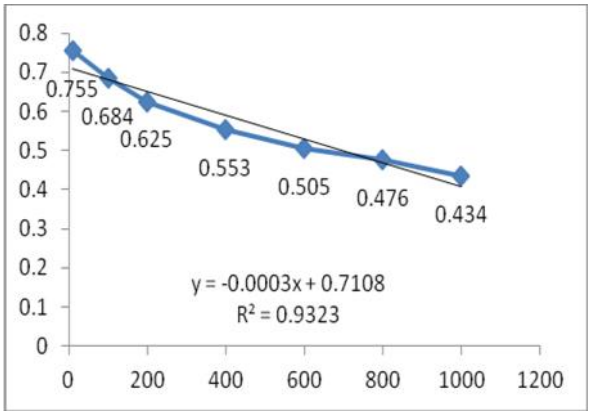
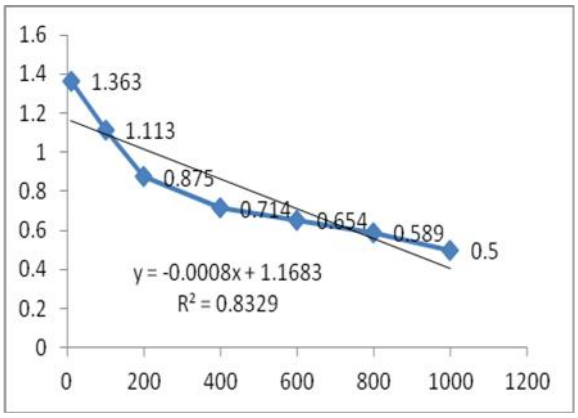


Arjuna Bark

Concentration	O.D	Inhibition Concentration
10	**0.333**	**1.363**
100	**0.291**	**1.113**
200	**0.251**	**0.875**
400	**0.224**	**0.714**
600	**0.214**	**0.654**
800	**0.203**	**0.589**
1000	**0.188**	**0.500**



RESULTS AND DISCUSSION :

The anti-oxidant activity of the various dosage forms has been done by DPPH radical scavenging assay method and Lipid peroxidation method. The IC-50 (Inhibitory Concentration) of the sample using DPPH method is tabled below, Tablet exhibited to have highest activity followed by Powder. The samples were compared with standard readings obtained of Vitamin-C.

S.No	PRODUCT	DPPH ASSAY (IC-50)
1	TABLET	29.87
2	CHOORNA	14.64
3	CRUDE BARK	15.86
4	CAPSULE	15.96
5	POWDER	20.6

The anti-oxidant activity of samples by Lipid peroxidation technique, prior to which rat liver was isolated and the protein content was determined and found to be 43µl i.e. 500 mitochondria in 1ml of solution. The samples were compared with standard readings cbtained of Vitamin-E. The Inhibition concentration of the samples using Lipid Peroxidation method is tabled below, Bark powder exhibited to have higher activity followed by choornem and capsule.

All the samples showed better activity on cell tissue culture rather than the DPPH radical scavenging activity.

S.no	Product	Lipid Peroxidation % Inhibition
1	**Tablet**	**40.23**
2	**Choornam**	**54.62**
3	**Bark**	**82.02**
4	**Capsule**	**47.64**
5	**Powder**	**44.70**

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SUMMARY AND CONCLUSION

Qualitative assessment of antioxidant potential of various Dosage forms of *Terminalia arjuna* has been performed.

The Antioxidant activity of the samples were determined by DPPH radical scavenging method and Lipid Peroxidation method. The IC 50 of the samples using DPPH methodwas performed and Tablet exhibited to have higher concentration followed by Powder. The samples were compared with readings of Vitamin-C.

The Antioxidant activity of the samples has done using Lipid peroxidation technique, prior to which rat liver was isolated and the protein content was determined and found to be 43µl i.e. 500 mitochondria in 1ml of solution. The samples were compared with standard readings obtained of Vitamin-E. The Inhibition Concentration of the samples using Lipid peroxidation method is tabled below, Bark powder exhibited to have highest concentration followed by Choornam and Capsule. All the samples showed better activity on the cell tissue culture rather than the DPPH radical scavenging activity.

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