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PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF TINOSPORA CORDIFOLIA

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Abstract:

The present study was undertaken to investigate in vitro antioxidant activity of aqueous and hydroalcoholic extract of whole plant of Tinospora Cordifolia. Family- Scrophularaceae. The total Phenolic content was determined using folin ciocalteau method while the total flavanoid content was determined using aluminum chloride method. In vitro antioxidant activity was evaluated using the Reducing power assay , Hydrogen peroxide scavenging assay, nitric oxide scavenging activity, superoxide scavenging activity and hydroxyl radical scavenging activity. The hydroalcoholic extract had more phenol concentration (116.1 mg/g of extract) when compared to aqueous extract (58 mg/g of extract). The flavanoid content was more in hydroalcoholic extract (242.6 mg/g of extract) when compared to that of aqueous extract (202.8 mg/g of extract). The reducing power and hydrogen peroxide scavenging of the extract was found to be concentration dependent. The nitric oxide scavenging activity . superoxide scavenging activity and Hydroxyl radical scavenging activity was also concentration dependent with IC_{50} value being 254.70 µg/ml , 934.06 µg/ml and 510.60 µg/ml respectively for Aqueous extract and 169.22 µg/ml , 495.83 µg/ml , 488 µg/ml respectively for hydroalcoholic extract. The order of the antioxidant potency of the whole plant extract is Hydroalcoholic >> aqueous. The results clearly indicate that aqueous and hydroalcoholic extract of Tinospora Cordifolia. has anti oxidant property which may be due to the presence of phenols and flavanoids.

Keywords: *Tinospora Cordifolia. ,Superoxide Scavenging Activity,Hydroxyl Radical Scavenging Activity,Hydrogen Peroxide Scavenging Activity .*

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INTRODUCTION:

Oxygen is an indispensible element for the sustenance of living beings and many biological systems. Cells reduce oxygen and generate adenosine triphosphate (ATP) in the mitochondria. By products known as free radicals are created during this process. These free radicals are beneficial in moderate levels but at higher concentrations can damage tissues by oxidative stress. Since more than half a century the deleterious effects of these reactive species are known but in the last two decades a lot of work has been done in this area. The important role played by anti oxidants in providing protection cannot be underestimated. Antioxidants are increasingly being used to prevent and also repair the damage caused by these free radicals.

A free radical may be defined as a molecule or molecular fragment containing one or more unpaired electrons in its outermost atomic or molecular orbital. These when formed can be highly reactive and can start a chain reaction. [1] The sources of free radicals can be endogenous and exogenous in nature. Endogenous sources of free radicals are intracellularly generated from auto-oxidation or inactivation of small molecules. Exogenous sources of free radicals are tobacco smoke, certain pollutants, organic solvents, anesthetics and pesticides. The sites of free radical generation encompass all cellular constituents including mitochondria, lysosomes, peroxisomes, endoplasmic reticulum, plasma membrane and sites within the cytosol. [2] Apart from this, certain medications metabolized to free radical intermediate products also cause oxidative damage within the target tissues. Exposure to radiation results in the formation of free radicals within the target tissues3.

Tinospora Cordifolia has been used in the Ayurvedic system of medicine for centuries. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration, [4] and to provide relief to patients with anxiety or epileptic disorders. [5] The plant has also been used in India and Pakistan as a cardiac tonic, digestive aid, and to improve respiratory function in cases of broncho constriction. [6] Recent research has focused primarily on *Tinospora Cordifolia* cognitive-enhancing effects, specifically memory, learning, and concentration and results support the traditional Ayurvedic claims. Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome, and gastric ulcers also supports the Ayurvedic uses of *Tinospora Cordifolia*. *Tinospora Cordifolia* antioxidant properties may offer protection from free radical damage in cardio vascular disease and certain types of cancer.

METHODS AND MATERIALS:

Collection and authentication of plant material:

The plant material (whole) i.e *Tinospora Cordifolia* was collected in the month of August 2022 from a local dealer Shantanu (9437066720), Subarnarekha marketing pvt ltd. , P.O jaleswar, dst. Balasore, Pin-756032, Orissa. Around 2kgs of plant was collected. The plant material was taxonomically identified by Dr. S.K Mahmood, Department of Botany, Nizam University- Hyderabad and a specimen was deposited in their herbarium against issue of Voucher no: 52136

Preparation of powder:

The plant material of *Tinospora Cordifolia* were shade dried and then powdered with a mechanical grinder to form a coarse powder. The powder was passed through sieve no 40 and was stored in an air tight container until further use. The powder was used for the extraction process.

Aquesous extract:

The aqueous extract of the plant was prepared using Maceration process. The coarde powder of plant (70g) was taken in a beaker with the water quantity of 1000ml and was macerated for 72 hours. During the maceration ocassional stirring and warming were carried out. After 72 hours , the suspension was filtered through a fine muslin cloth. The solvent was removed by heating it and a greenicsh black residue was obtained. (Yield: 9.14% w/w w.r.t to dried plant material). The dried aqueous extract was stored in a dessicator till needed.

Hydro Alcoholic extract:

The hydro alcoholic extract of *Tinospora Cordifolia* was obtained from a private herbal industry named AMSAR GOA PRIVATE LIMITED which is located in Goa. A sample extract (40g) was given (Batch no : L/11011(May 2011) which was extracted using Soxhlet apparatus . The solvent used was a hydro alcholic i.e a mixture of Water and Alcohol (ethanol) in the ratio 60:40 % v/v. The extract was collected and stored till needed. The reciept no is AGPL/096/2011-12.

Chemicals required:

Potassium ferric cyanide , trichloro acetic acid , ferric chloride, sodium dihydrogen phosphate , disodium hydrogen phosphate, hydrogen peroxide , Deoxyribose , EDTA , Ascorbic Acid , thiobarbituric acid , trishydrochloride , sodium dodecyl sulfate , acetic acid (glacial) , butanol , pyridine , ammonium

ferrous sulphate, sodium nitropursside , sulfanilic acid , N-(1-Naphthyl)ethylenediamine dihydrochloride , Dimethyl sulphoxide , NBT , sodium hydroxide , Gallic acid , sodium carbonate , folin ciocalteau reagent , Aluminium chloride , sodium nitrite , catechin , distilled water etc.

Essential instruments:
UV- VISIBLE Spectrophotometer, pH meter, Incubator, homogenizer, water bath, heating mantle, centrifuge, refrigerator, weighing balance etc.

Glassware:
Test tubes, conical flask, pipettes, beakers, stirrer, measuring cylinder, funnel, centrifuge tubes, Reagent bottles etc. Test tube stand, test tube holders, filter paper, butter paper, spatula, thermometers, stands, tissue paper, zip pouches, markers, gloves, labels, cotton swabs, disinfectant etc.

Phytochemical evaluation:
500 mg of the dried extract were reconstituted in 10 ml of respective solvents and used for preliminary phytochemical testing for the presence of different chemical groups of compounds. Carbohydrates,Glycoside,Saponins, Alkaloids Phytosterols,Fixed Oils, Gums and Mucilage, Proteins, Phenolic compounds and Tannins, Flavonoids

Determination Of Total Phenolic Content: [7]
Total Phenolic content of the extract was determined by Folin ciocalteau reagent according to Singleton and Rossi using Gallic acid as a standard. 0.1ml (100 µg) of sample solution was made up to 3ml using distilled water. About 0.5ml of Folin ciocalteau reagent was added and mixed thoroughly. Incubated for 3min at room temperature. After incubation 3ml of 20% Na_2CO_3 was added and mixed thoroughly, incubated in boiling water bath for 1 min. the absorbance was measured at 650nm. The concentration of total phenols was expressed in terms of mg of Gallic Acid equivalents per gram of extract.

Determination Of Total Flavanoid Content: [8]
Total Flavanoid assay was measured by the aluminum chloride colorimetric assay. An Aliquot (1ml) of extracts or standard solution of catechin (20, 40, 60, 80 and 100µg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To the flask was added 0.3ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6th min, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled H_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/ g of extract. Samples were analyzed in duplicates.

In vitro antioxidant activity:
1) ferric reducing power: [9]
The reducing power was determined according to the method of Oyaizu. Different concentrations of the extract (50, 100 ,150 ,200, 250 µg/ml) prepared in methanol were mixed with phosphate buffer (2.5 ml , 0.2M, pH 6.6) and potassium ferric cyanide { $K_3Fe(CN)_6$} (2.5ml , 1%) . The mixture was incubated at 50°C for 20 min and 2.5ml of tricholoroaceticacid (10%) was added to the mixture ,which was then centrifuged at 3000rpm for 10min. the upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml)and $FeCl_3$ (0.5ml , 0.1%) and the absorbance was measured at 700nm. Increased Absorbance of the reaction mixture indicated increased reducing power. Ascorbic Acid was used as Standard.

2) Hydrogen Peroxide Scavenging Activity: [10]
The H_2O_2 scavenging ability of the extract was determined according to the method of Ruch et al. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). 100, 200,300,400,500 µg/ml concentrations of extract in 3.4ml Phosphate buffer were added to H_2O_2 solution (0.6ml, 40mM). The absorbance value of the reaction mixture was recorded at 230nm. The percent of scavenging of H_2O_2 was calculated by using the following equation.
% of scavenging = [(A of control – A of sample) / A of Control] X 100
Where A of control is the absorbance of the control reaction (containing all reagents except test compound) and a sample is the absorbance of the test compound. Test was carried out in triplicate.

3) Nitric Oxide Scavenging Activity: [11]
Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1%

w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

The nitric oxide radicals scavenging activity was calculated according to the following equation:
% Inhibition = [(A0-A1) / A0] × 100)

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

4.) Superoxide radical scavenging activity: [12]
To the reaction mixture containing 0.1 ml of NBT (1 mg/ml solution in DMSO) and 0.3 ml of the extracts, the compound and standard in dimethyl sulphoxide (DMSO), 1 ml of alkaline DMSO (1 ml DMSO containing, 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm.

% inhibition = Absorbance of Control – Absorbance of Test/ Standard X 100
Absorbance of Control

5) Hydroxyl Radical Scavenging Activity: [13]
The degradation of deoxyribose generated by Fenton reaction was measured spectrophotometrically in the presence and absence of test compound. The final reaction mixture in each test tube consisted of 0.3 ml each of Deoxyribose (30 mM), ferric chloride (1mM) , EDTA (1mM), Hydrogen peroxide (20mM), in the phosphate buffer having pH 7.4 and 0.3 ml of test compound at different concentrations. The test tubes were incubated for 30 min at 37°C after incubation , trichloro acetic acid (0.5 ml , 5%) and thiobarbituric acid (0.5 ml , 1%) were added and the reaction mixture was kept in boiling water bath for 30 min. it was then cooled and the absorbance was measured at 532 nm. The results were express as a % of scavenging of hydroxyl radical.

6) Lipid Peroxidation Inhibiting Activity: [14]
To 0.1ml of rat liver homogenate (25% w/v) in tris-Hcl buffer (40 mM, pH 7.0), 0.1ml each of KCL (30 mM), ascorbic acid (0.06mM) and ferrous ion (0.16 mM) and various quantities of extracts and ascorbic acid was incubated for 1 hr at 37°c. The reaction mixture (0.5 ml) was treated with sodium dodecyl sulfate (0.2 ml, 8.1%), thiobarbituric acid (1.5 ml, 0.8%) and acetic acid (1.5 ml, 20%, pH 3.5). The total volume was then made up to 4 ml by adding distilled water and kept in oil bath at 100°c for 1 hr. After the mixture had been cooled 1 ml distilled water and 5 ml of butanol-pyridine mixture (15:1 v/v) was added. Following vigorous shaking, the tubes were centrifuged and the absorbance of the organic layer containing the chromophore was read at 532 nm.

RESULTS AND DISCUSSIONS:

Preliminary phytochemical screening:

Table-1

Tests for	Aqueous extract	Hydro alcoholic extract
Carbohydrates	+	+
Glycoside	+	+
Saponins	+	+
Alkaloids	-	-
Phytosterols	-	-
Fixed Oils	+	+
Gums and Mucilage	-	-
Proteins	+	+
Phenolic compounds and Tannins	-	-
Flavonoids	+	+

Data showing the preliminary phytochemical screening of aqueous and hydro alcoholic extract of *Tinospora Cordifolia.* (+ = present - = absent)

Estimation of total phenolic content:

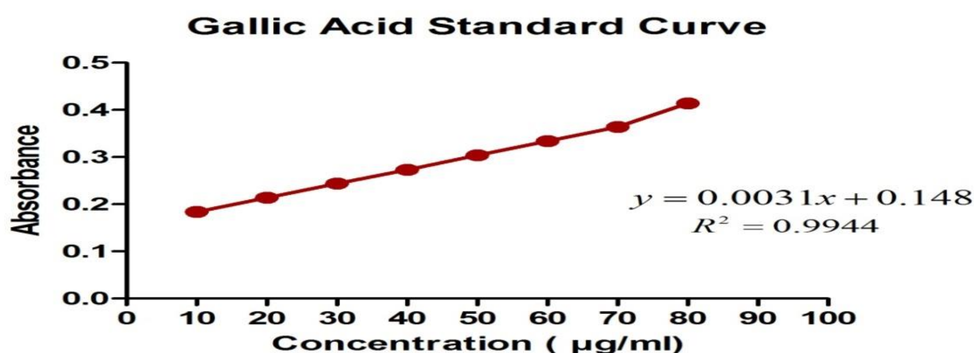
Table 2: Data showing absorbance of various concentration of Gallic acid

Standard (Gallic acid) Calibration curve	
Concentration (µg/ml)	Absorbance
10	0.184
20	0.214
30	0.244
40	0.273
50	0.304
60	0.334
70	0.364
80	0.414

Sample	
Concentration (100µg/ml)	Absorbance
Aqueous Extract	0.166
Hydro alcoholic extract	0.184

TOTAL PHENOLIC CONTENT:

Graph -1



From the Standard Graph of Gallic Acid, The total phenol concentration present in the Aqueous and Hydro alcoholic extract was found to be:
Aqueous Extract: 58 mg GAE/ g of extract
Hydro Alcoholic Extract: 116.1 mg GAE / g of extract

Total flavanoid content:

Table 3: Data showing absorbance of various concentration of Catechin.

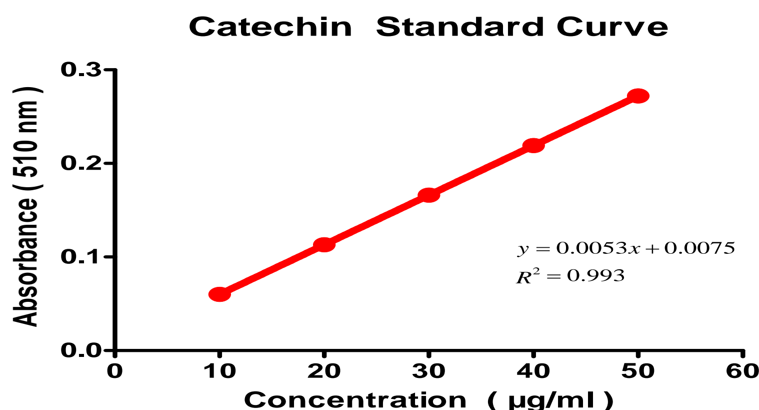
Catechin Standard curve	
Concentration (µg/ml)	Absorbance
10	0.060
20	0.113
30	0.166
40	0.219
50	0.272

Sample Solution	
Aqueous Extract (100 µg/ml)	0.138
Hydro Alcoholic Extract (100 µg/ml)	0.115

Graph -2

From the Standard Graph of Catechin, The total flavanoid concentration present in the Aqueous and Hydro alcoholic extract was found to be:

Aqueous Extract: 202.8 mg of CE/ g of extract



Hydro alcoholic Extract: 242.6 mg of CE / g of extract

1)FERRIC REDUCING POWER:

Table:4

Extract	50	100	150	200	250
Ascorbic acid(std)	0.743±0.001	0.787±0.002	0.811±0.0015	0.817±0.0015	0.826±0.0025
AQUEOUS	0.143±0.0014	0.152±0.0025	0.164±0.0015	0.171±0.003	0.222±0.003
HYDROALCHLC	0.202±0.002	O.244±0.004	0.296±0.006	0.323±0.003	0.340±0.004

Results expresses as mean±sem for six observations

2) HYDROGEN PEROXIDE SCAVENGING ACTIVITY:

Table 5:

EXTRACT	100	200	300	400	500
ASCROBIC ACID	0.769±0.001	0.783±0.001	0.823±0.002	0.830±0.002	0.835±0.003
AQUEOUS	0.392±0.002	0.502±0.003	0.653±0.002	0.679±0.004	0.770±0.005
HYDROALCHOLIC	0.627±0.002	0.711±0.004	0.756±0.003	0.785±0.002	0.817±0.002

Results expresses as mean±sem for six observations

3) NITRIC OXIDE SCAVENGING ACTIVITY:

Table 6:

EXTRACT	25	50	75	100	125	IC50(µg/ml)
Ascorbic acid	8.7±0.19	11.69±0.31	13.63±0.6	23.48±0.4	32.30±0.6	210.75
Aqueous	14.97±0.7	26.04±0.7	27.69±0.3	28.61±0.3	30.76±0.3	254.70
Hydroalcholic	11.17±0.4	18.86±0.7	28.30±0.6	32.61±0.9	36.91±0.6	169.22

Results expresses as mean±sem for six observations

4)SUPEROXIDE RADICAL SCAVENGING ACTIVITY:

Table7:

Extract	100	200	300	400	500	Ic50(µg/ml)
Ascorbic	24.99±0.6	33.32±0.6	38.88±0.6	44.44±0.6	54.86±1.39	456.57
Aqueous	21.52±0.6	28.68±0.3	30.63±0.6	33.56±0.3	35.52±0.53	934.06
Hydroalcholic	26.38±2.5	32.86±1.0	37.96±1.74	42.82±2.23	51.47±6.27	495.83

Results expresses as mean±sem for six observations

5) HYDROXYL RADICAL SCAVENGING ACTIVITY:

Table 8:

Extract	100	200	300	400	500	Ic50(µg/ml)
Ascorbic	29.54±0.4	34.94±2.2	40.34±0.5	45.85±0.9	54.80±1.9	448.19
Aqueous	15.51±1.25	21.56±1.26	25.16±0.6	42.13±1.2	51.18±0.4	510.60
Hydroalcholic	23.29±0.5	24.99±0.5	29.32±1.1	43.64±1.2	54.16±1.5	488.1

Results expresses as mean±sem for six observations

6) LIPID PEROXIDATION INHIBITING ACTIVITY:

Table 9:

Extract	100	200	300	400	500
Ascorbic	0.134±0.001	0.144±0.004	0.190±0..3	0.237±0.002	0.285±0.002
Aqueous	0.87±0.001	0.102±0.002	0.126±0.001	0.139±0.001	0165±0.002
Hydroalcholic	0.092±0.002	0.123±0.001	0.170±0.002	0.222±0..2	0.243±0.002

Results expresses as mean±sem for six observations

From the (table-1),the phytochemical screening of aqueous and hydro-alcholic extract of *Tinospora Cordifolia* we come to know that aqueous extract and hydroalcholic contain carbohydrates,glycosides,alkaiods,flavanoidand phenols but proteins ,steroids and tannins were absent.

From the (table-2 and graph -1) the total phenol concentration present in the aqueous contain 58mg GAE/g and hydroalcoholic extract contain 116.1 mg GAE/g .

From the (table-3 and graph-2) the total flavanoid concentration present in the aqueous extract is 202.8mg of CE/g and hydroalcholic extract contain 242.6 mg of CE/g.

From the table:4 :The reducing power has been used as one of the important antioxidant capabilities for medicinal herbs. The reducing power of Aqueous and Hydro alcoholic extract of *Tinospora Cordifolia* was dose-dependent. The absorbance increases with increase in the concentration. From the above (table-3) it can be inferred that the increase in ferric reducing activity was more for hydro alcoholic extract of *Tinospora Cordifolia* then the aqueous extract From the (table-5)_Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects it is therefore biologically advantageous for cells to control the amount of H_2O_2 that is allowed to accumulate. As shown in the above graph, the aqueous and hydro alcoholic extract of *Tinospora Cordifolia* has demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. The decomposition of H_2O_2 by the extract may at least partly result from its antioxidant and free radical scavenging activity. The activity was higher for hydro alcoholic when compared to aqueous and was comparable to that of standard i.e. ascorbic acid.

From (table-6)_Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO_2, N_2O_4, N_3O_4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of

this damage. Aqueous and Hydro alcoholic extract of *Tinospora Cordifolia* shows increase in nitric oxide, as shown in the above figure. The action was dose dependent. The values were comparable to that of the standard i.e. ascorbic acid.

From (table-7)Superoxide radicals are known to be very harmful to the cellular component. Super oxide free radical was formed by alkaline DMSO which reacts with NBT to produce colored diformazan. The Aqueous and Hydro alcoholic extract of *Tinospora Cordifolia* scavenges super oxide radical and thus inhibits formazan formation. The above graph illustrates increase scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the *Tinospora Cordifolia* extracts.IC_{50} value of ascorbic acid is 456.57 µg/ml. The IC_{50} value of Aqueous and Hydro alcoholic extract of *Tinospora Cordifolia* is 934.06 µg/ml and 495.83 µg/ml respectively. From the above graph it can be inferred that the hydro alcoholic extract has better super oxide scavenging ability when compared to aqueous and the values are comparable with that of standard.

From (table-8) Hydroxyl radical is highly reactive oxygen centered radical formed from the reaction of various hydro peroxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acids in membranes and most biological molecule it contacts and is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxidic reaction of lipids. Activity of the aqueous and hydro alcoholic extract of *Tinospora Cordifolia* on hydroxyl radical has been shown in above figure..The plant extract exhibited concentration dependent scavenging activity against hydroxyl radial generated in a Fenton reaction system. Hydro alcoholic extract has better scavenging activity on hydroxyl radical when compared to aqueous extract. The IC_{50} value of ascorbic acid is 448.19 µg/ml. the IC_{50} value of aqueous and hydro alcoholic extract is 510.60 µg/ml and 488.00 µg/ml respectively.

From (table-9) The lipids in membrane are continuously subjected to oxidant challenges. Oxidant induced abstraction of a hydrogen atom from an unsaturated fatty acyl chain of membrane lipids initiates the process of LPO, which propagates as a chain reaction. In the process, cyclic peroxides, lipid peroxides and cyclic end peroxides are generated, which ultimately are fragmented into aldehydes like MDA. MDA forms a pink chromogen with TBA that absorbs at 532 nm. Bacopa monnieri aqueous and hydro alcoholic extract inhibited the amount of MDA generated (and thus lipid per oxidation) in liver homogenate which is presented in above graph. Thus, the decrease in the MDA level in the extracts indicates the role of the extracts as an antioxidant where hydro alcoholic extract showed higher inhibition than the aqueous extract.

CONCLUSION:

Antioxidants were believed to be a panacea for many disorders in the early years of their discovery. Their importance still remain the same even several years later , when their discovery has been superseded by numerous noteworthy contributions.

The phytochemical screening of aqueous and hydro alcoholic extracts of *Tinospora Cordifolia* showed the presence of flavanoids and phenols which are considered to be responsible for antioxidant activity. Therefore *Tinospora Cordifolia* was considered to possess antioxidant activity.

The literature clearly suggests that *Tinospora Cordifolia* has been widely used as potent antioxidant as demonstrated in ethno medicine. In order to evaluate the veracity of the traditional use of *Tinospora Cordifolia*, in vitro antioxidant activity of aqueous and hydro alcoholic extracts of *Tinospora Cordifolia* were conducted.

The investigations on *Tinospora Cordifolia* plant extracts were found to yield substantial positive data pointing towards the evidence of antioxidant activity. The data obtained from ferric reducing power, hydrogen peroxide radical assay, nitric oxide radical scavenging , superoxide radical scavenging and microsomal lipid peroxidation assay clearly suggested that the antioxidant activity of *Tinospora Cordifolia* was dose dependent. It can also be noted that the extract of *Tinospora Cordifolia* was found to scavenge the free radicals such as peroxides, superoxides and hydroxyl radicals. From the various test it was concluded that the hydro alcoholic extract of *Tinospora Cordifolia* is found to possess greater antioxidant potential when compared to that of aqueous extract.

Finally our studies concluded that *Tinospora Cordifolia* has antioxidant activity and therefore it can be used as an antioxidant along with the other suggested and proven therapeutic remedies such as nervine tonic, cardiotonic, cognitive enhancer and alterative.

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