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Evaluation of Antioxidant and Anti-Hyperlipidemic Effect of Tridax Procumbens L. Flower Extract by in Vitro Method

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ABSTRACT

This study assessed the *in vitro* antioxidant and anti-hyperlipidemic properties of *Tridax procumbens Linn* methanolic and hydro alcoholic flower extract. The amount of antioxidant activity was measured using techniques including DPPH (2, 2-diphenyl-2-picryl hydroxyl) and nitric oxide. We analyzed the percentage of different free radicals that were scavenged using ascorbic acid, a common antioxidant. Using the TBARS assay and lipid peroxidation measurements, the extracts anti-hyperlipidemic potential was also assessed. Furthermore, the phytochemical analysis of the floral extracts that were methanolic and hydroalcoholic revealed the presence of flavonoids, phenols, saponins, terpenoids, triterpenes, alkaloids, glycosides, and tannins in substantial amounts. Significant antioxidant activity was found ($P<0.001$) for DPPH scavenging at various flower extract doses. The methanolic extract's IC50 value and % inhibition were higherthan hydro-alcoholic extracts.

Keywords: Tridax Procumbens, Flower Extract, In -Vitro, Antioxidant, Anti-Hyperlipidemic

INTRODUCTION

Antioxidants are substances that delay or inhibit the oxidation of substrates, protecting the bio membrane against reactive oxygen species (ROS)-mediated tissue damage. They provide electrons that neutralize free radicals, which can cause degenerative and life-threatening diseases [8]. Primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent their formation. Antioxidant compounds like phenolic acids, polyphenols, and flavonoids scavenge free radicals, inhibiting oxidative mechanisms leading to degenerative diseases [1][5]. Many natural antioxidants are plant-based and belong to the phenolic and polyphenolic class, as well as carotenoids and antioxidant vitamins [5]. Synthetic antioxidants like butylated hydroxyl toluene and butylated hydroxy anisole have been reported to be dangerous for human health. The search for effective, nontoxic natural compounds with antioxidative activity has intensified in recent years.

Hyperlipidemia, characterized by elevated levels of plasma lipid, serum cholesterol, and triglyceride, is a significant risk factor for coronary heart disease (CHD). Primary hyperlipidemia can be treated with

drugs, while secondary types originating from diabetes, renal lipid necrosis, or hypothyroidism require treatment of the original disease. Excessive intake of exogenous TG and cholesterol is associated with an elevated risk of atherosclerotic disease, a lipid storage disease involving arterial cholesterol deposition, endothelial injury, and platelet aggregation. Levels between 200 and 240 mg/dL indicate moderate risk, while levels exceeding 240 mg/dL indicate high risk. The complex interaction of these three types of lipids contributes to hyperlipidemia. Many medicinal plants have active principles useful for treating diseases, with some having hyperlipidemic action and other beneficial properties like antianginal and antiplatelet actions. These plants contain compounds that work synergistically on multiple parts of the body.

The study focuses on the antioxidant and antihyperlipidemic effects of *Tridax procumbens* flowers, a medicinal plant known for its pharmacological activities. The extracts were prepared using Soxhlet extraction methods, with the methanolic fraction extracted with petroleum ether, diethyl ether, and ethylacetate. The ethyl acetate fraction was hydrolyzed with 7% H2SO4 and extracted with ethyl acetate to obtain crude Quercetin. Quercetins are antioxidants that scavenge free radicals, damaging cell membranes and DNA. Antioxidant activity was assessed using DPPH and nitric oxide radical inhibition methods, while antihyperlipidemic activity was assessed using lipid peroxidation and TBARS assay methods.

MATERIALS AND METHODS

Sample Collection

The plant *Tridax procumbens* are widely found throughout India along road sides and waste places. The flowers for the evaluation were collected from roadsides, wastelands, home courtyard and crops in Erattayal, Palakkad.

Chemical and Reagents

Methanol, Ethanol (95%),Toluene, Ethyl acetate, Formic acid, Silica Gel G, Vanillin, Sulphuric acid, Dragendroff's reagent, Mayer's reagent, Hager's reagent, Wagner's reagent, Molish reagent, Fehling's reagent A, Fehling's reagent B, Benedicts reagent, Sodium hydroxide, Vanillin HCl , Chloroform, Ammonia, Lead acetate (10%), Ferric chloride in 90% alcohol, Gelatin (1%), Sodium chloride (10%), Conc. Sulphuric acid, Acetic anhydride, DPPH reagent, Petroleum jelly. All other chemicals and reagents used in this study were of analytical grade, Sodium nitroprusside, Griess reagent, Nitric acid.

Authentication of plant

The authentication of the plant extract of *Tridax Procumbens* was done byDr. M. Bheemalingappa, Scientist B, Forest Botany Department, KSCSTE-Kerala Forest Research Institute, Peechi ,Peechi (p.o) , Thrissur.

Extraction of *Tridax procumbens Linn* flower

In Soxhlet Extraction,

All the plant materials were dried and finely powdered. The finely powdered raw materials were weighed and rolled in a filter paper and kept in the condenser part of the Soxhlet apparatus. Then, add methanol in the condenser. Add a porcelain piece in to the round bottom flask in order to avoid the bombardment. Then the extraction process continuous for 72 hours. Then the extract was collected and dried in a hot air oven and kept in the refrigerator.

$$\%\text{Yield} = (\text{Practical yield}/\text{Theoretical yield})*100$$

= (3.938/50)*100
=7.8760%

In Simple Maceration,

The finely powdered raw materials are placed in an iodine chamber. Then add hydro-alcoholic solution (Ethanol: Water in the ratio of 1:1) in to the chamber. The mixture is subjected to continuous agitation for 3 days and filtered and dried. Finally, the crude extract was collected.

%Yield = (Practical yield/Theoretical yield)*100
= (4.324/30)*100
=14.4133%

Preparation of the Test solution: The flower extract were diluted to suitable concentrations with the help of distilled water.

Preliminary Phytochemical Screening:

Table 1: Qualitative phytochemical analysis of *Tridax procumbens* flower extracts

Sl. No.	Phytoconstituents	Inference
1	Carbohydrates	-
2	Alkaloids	+
3	Saponins	+
4	Steroids	+
5	Triterpenoids	+
6	Tannins	+
7	Flavonoids	+

Thin Layer Chromatography (TLC) analysis:

The process which is commonly known as thin layer chromatography, a solute is distributed in between two phases: a liquid mobile phase and a stationary phase that acts on the principle adsorption. The adsorbent is a thin, uniform layer of dry finely powdered material which is applied to a glass, plastic or metal sheet or plate. Among these, glass plates are most commonly used. Based on partition and adsorption, separation can also be accomplished, depending on the specific kind of support, how it is prepared, and how it is used with various solvents. By comparing spots acquired with an unknown and reference sample chromatographed on the same plate that have the same Rf value and almost equal magnitude, one can identify the samples. A visual comparison of the size and intensity of the spots usually serve for semi quantitative estimation.

Antioxidant activity

Anti-oxidants help the body to get rid of oxygen free radicals, which are thought to contribute to cancer development by damaging the DNA. Many plants and their extracts are rich sources of agents such as anti-oxidants, which can prevent the occurrence of cancer by reducing free-radical induced cell damage. A majority of disease conditions like hypertension, hyperlipidemia ,atherosclerosis, ischemic disease, Alzheimer's disease, Parkinson's diseases, cancer and inflammatory are being caused primarily due to the imbalance between pro-oxidant and antioxidant homeostasis[9].

In the present study, the antioxidant and antihyperlipidemic activities of the flower extract were carried out, based on the literature survey.

In vitro Measurement of Antioxidant Properties

2,2-Diphenyl-1-Picrylhydrazyl (DPPH)Radical Scavenging Activity Assay

The antioxidant activity of various concentrations of flower extracts of *Tridax procumbens* was assessed on the basis of the radical scavenging effect of the stable DPPH free radical. The DPPH free radical is reduced to a corresponding hydrazine when it reacts with a hydrogen donor.

The DPPH radical is purple in colour and upon reaction with hydrogen donor, it changes to yellow in colour. It is a discoloration assay, which is evaluated by the addition of antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured. It shows a characteristic absorption at 517 nm. Antioxidant react with DPPH and converts it to 1,1- diphenyl- 2- picryl- hydrazine and the degree of discoloration indicates the scavenging activity of the drug[24].

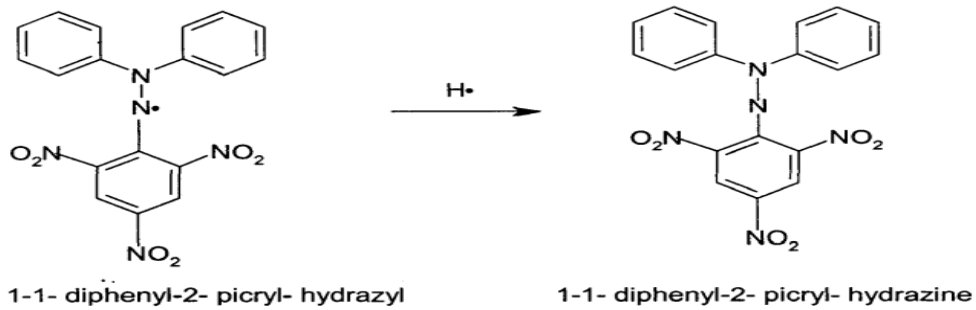

Fig 1: Reaction mechanism of free radical scavenging assay

The free radical scavenging activity of the samples was determined according to the method. A freshly prepared solution of 4mg of DPPH in 100ml methanol was used for the UV measurements. The samples of different concentrations (20, 40, 60, and 80,100,120) were added to DPPH solution. Then, it was allowed to take place in the dark at room temperature. Ascorbic acid was used as a standard. The inhibition percentage of DPPH radical scavenging activity was calculated using the following equation. (57)

$$\textbf{Inhibition (\%)} = [(A0 - A)/A0] \times 100$$

Where, A0 is the absorbance of DPPH in the absence of the sample and A is the absorbance of DPPH in the presence of the sample.

The IC50 values (the concentration required to scavenge 50% of the free radical) were estimated from a plot of % inhibition against the concentration of the sample solutions.

Nitric Oxide (NO) Radical Scavenging Assay

In order to perform the nitric oxide (NO) radical scavenging experiment, 0.6 mL of 10 mm sodium nitroprusside was combined with 1 mL of Tridax procumbens flower water extract at varying concentrations (4–64 µg/mL). After 150 minutes of incubation at 25°C, the mixture was mixed with 1.0 mL of Griess reagent, which had been previously made using 1% sulfanilamide, 0.1% naphthyl ethylene diamine dichloride, and 2% phosphoric acid. Ascorbic acid is used as standard. At 546nm,the absorbance was measured[25].

The inhibition was calculated by the following equation:

%inhibition of NO radical = [A0 − A1]/A0 × 100

Where, A0 is the absorbance before the reaction&A1 is the absorbance after the reaction has taken place with Griess reagent.

The decreasing absorbance indicates a high NO scavenging activity.

***Invitro* Measurement of Antihyperlipidemic activity**

***In vitro* Lipid Peroxidation (LPO) Assay**

Preparation of chicken liver homogenate

Take one lobe of the chicken liver and wash with 0.9% NaCl solution. Tissue homogenate was prepared in a ratio of 1 g wet tissue to 10 times (w/v) 0.05 M ice-cold phosphate buffer (pH-7.5) and homogenized by using Teflon homogenizer. The homogenate was used for the estimation of thiobarbituric acid reactive substances (TBARSs)

TBARS (Thiobarbituric Acid Reactive Substances) assay

The LPO of *Tridax procumbens* extract was determined by the method of Kumari et al. (2016). Liver homogenate (0.25 mL) was mixed with 0.1 mL Tris HCL buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL 4 mm FeCl2 solution and 0.05 mL of the test extracts. All extracts were tested at five different concentrations (4–64 µg/mL). The mixture was incubated at 37◦C for 1 h and then 1.5 mL 0.8% (w/v) 2- thiobarbituric acid, 1.5 mL 20% acetic acid, and 0.2 mL 8.1% (w/v) sodium dodecyl sulfate were added to the reaction mixture. The mixture was made up to 4.0 mL with distilled water and heated at 95◦C for 60 min. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added. The mixture was shaken vigorously. The absorbance was measured at 532 nm in a spectrophotometer (Beckman, UK). Ascorbic acid was taken as a standard[26].

RESULTS

Phytochemical analysis

Phytochemical analysis of flower extract of *Tridax procumbens* shows the presence of alkaloids, saponins, steroids, triterpenoids, flavonoids and tannins.

Thin Layer Chromatographic analysis

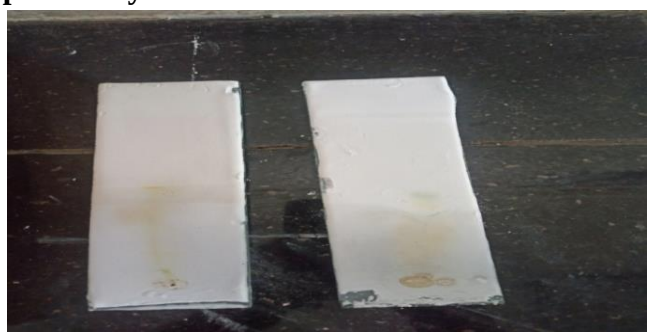

Fig 2: Development of the solvent front in TLC Plate

Rf Value = $\frac{distancetravelledbysolutefront}{distancetravelledbysolventfront}$

Rf value by soxhlet extraction method:

Rf value =11/15 = 0.7333

Rf value by simple maceration method:

Rf value =8/15 =0.5333

A TLC plate shows the presence of Flavonoids content appears in the solvent front.

Antioxidant activity

DPPH Radical Scavenging Activity

In DPPH method, IC_{50} values of methanolic flower extract of *Tridax procumbens* was found to be **88.64±1.0µg/ml** and IC_{50} values of hydro-alcoholic flower extract of *Tridax procumbens* was found to be **70.3 ±1.1 µg/ml**.

The standard drug ascorbic acid shows an IC_{50} value of **102.6±1.1µg/ml.**

These result shows that the methanolic flower extract of *Tridax procumbens* has higher antioxidant potential in scavenging free radicals than hydro alcoholic aqueous flower extract.

Nitric Oxide Radical Scavenging Activity

In nitric oxide method, methanolic flower extract of *Tridax procumbens* have an IC_{50} value of **66.8±1.1µglml** and Hydroalcoholic flower extract of Tridax procumbens was found to be **60.7±1.1µg/ml**.

The standard drug, ascorbic acid, shows an IC_{50} value of **102.6±1.1µglml.**

These results show that the methanolic flower extract of *Tridax procumbens* has a higher antioxidant potential.

Table 2: Antioxidant activity of methanolic and hydro alcoholic flower extracts by DPPH and nitric oxide methods

S. No.	Test Compounds	IC50 values ± SEM (µg/ml)	
		DPPH	Nitric oxide
1	Methanolic flower extract	88.64±1.0	66.8±1.1
2	Hydro-alcoholic flower extract	70.3±1.1	60.7±1.1
3	Ascorbic acid	102.6±1.1	102.6±1.1

SOXHLET EXTRACTION:

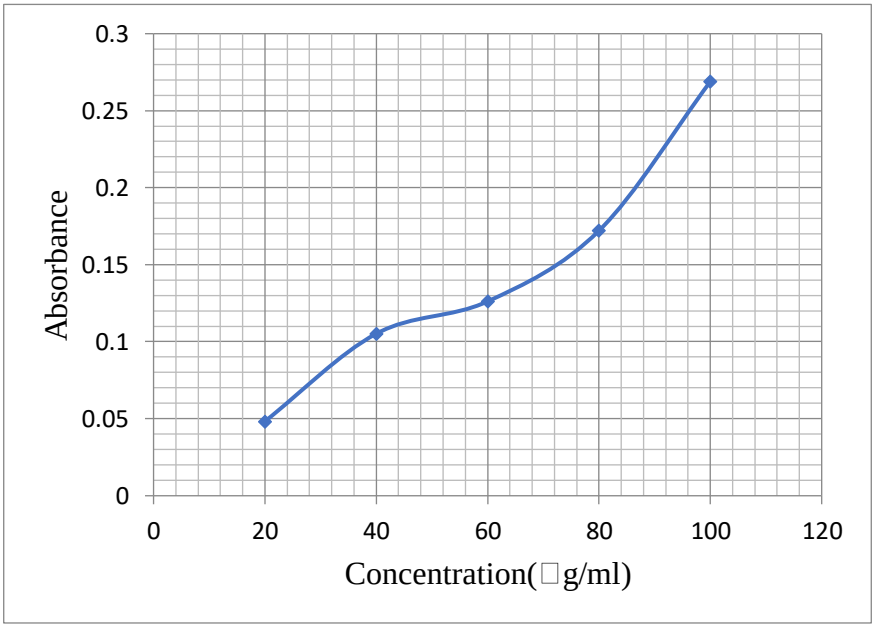

Fig 3: Graphical representation of absorbance obtained at various concentrations through Soxhlet Extraction

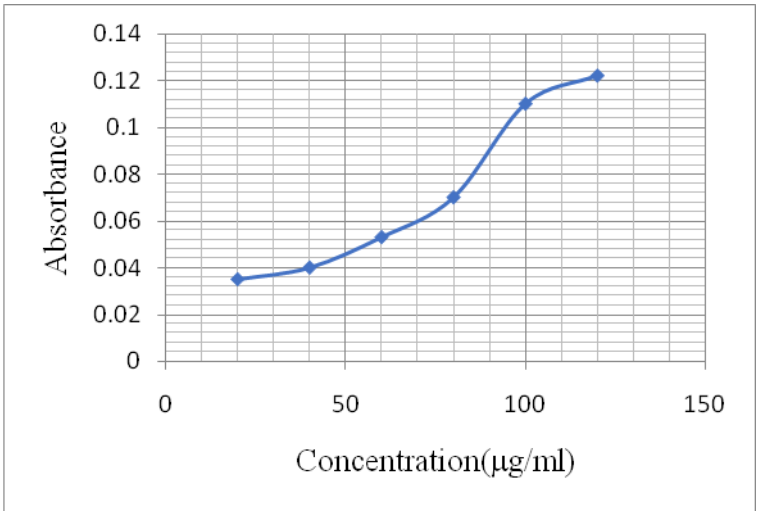

Fig 4: Antioxidant activity of the hydro –alcoholic flower extract of *Tridax procumbens* by nitric oxide methods

Table 3: Absorbance obtained at different concentration by Ascorbic acid, DPPH and Methanol through Soxhlet Extraction

Concentration (µg/ml)	Absorbance (Mean ± SEM)		
	Ascorbic acid (ml)	DPPH (ml)	Methanol (ml)
20	1	1.5	q.s to 3ml
40	1	1.5	q.s to 3ml
60	1	1.5	q.s to 3ml

80	1	1.5	q.s to 3ml
100	1	1.5	q.s to 3ml
120	1	1.5	q.s to 3ml

Inhibition (%) = [(A_0 − A)/A_0] × 100

For Soxhlet extraction: A_0=0.009

A=0.030

% Inhibition= [(0.009-0.030)/0.009]*100

= 233.3333%

For Simple maceration: A_0=0.028

A=0.024

% Inhibition= [(0.028-0.024)/0.028]*100

= 14.2857%

NITRIC OXIDE SCAVENGING ASSAY:

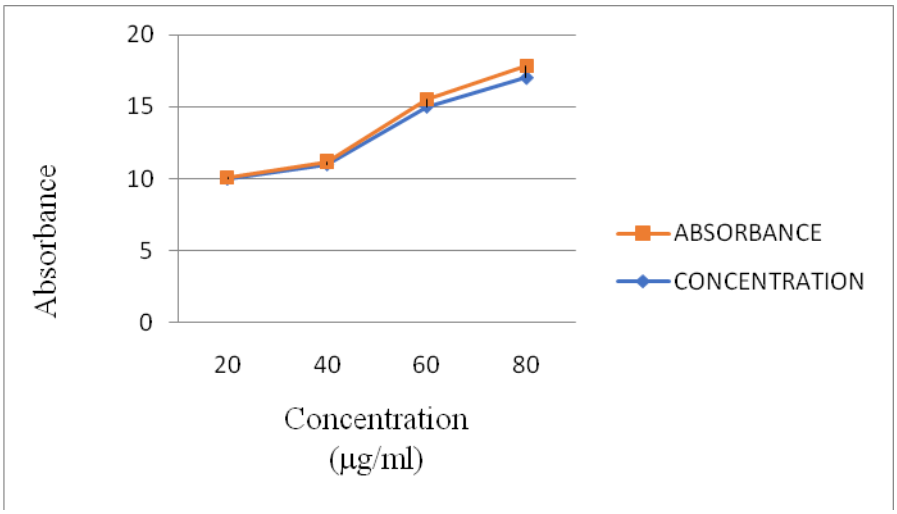

Fig 4: Graphical representation of absorbance at various concentrations through Nitric oxide scavenging assay

Percentage scavenging activity = [($A_{control}$ − A_{test} or A_{std})/$A_{control}$] *100

where $A_{control}$ is the absorbance of control and A_{test} or A_{std} is the absorbance of test or standard, respectively.

For Soxhlet Extraction:

A control=0.333 & A std =0.172

Percentage Scavenging Activity= [(0.333-0.172)/0.333]*100

=48.3483%

TBARS ASSAY:

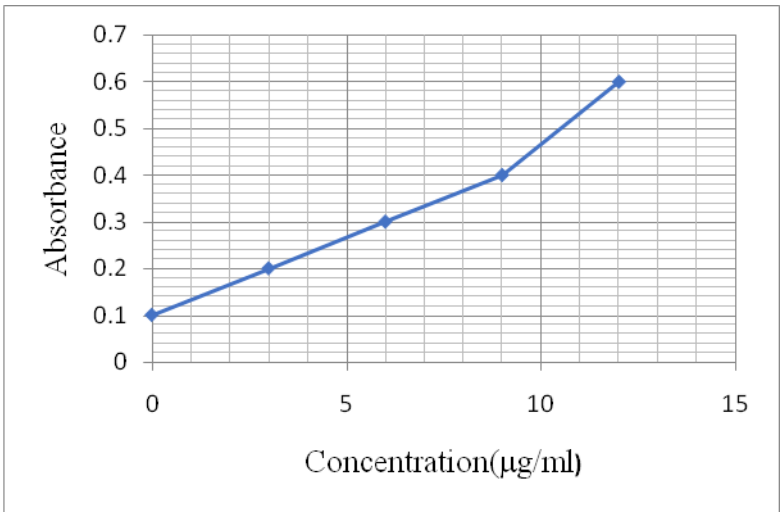

Fig 5: Graphical representation of absorbance at various concentration obtained through TBARS method

In the above graphical representations, Soxhlet extraction by methanol having the relevant results as compared to the Simple maceration or Hydroalcoholic extraction.

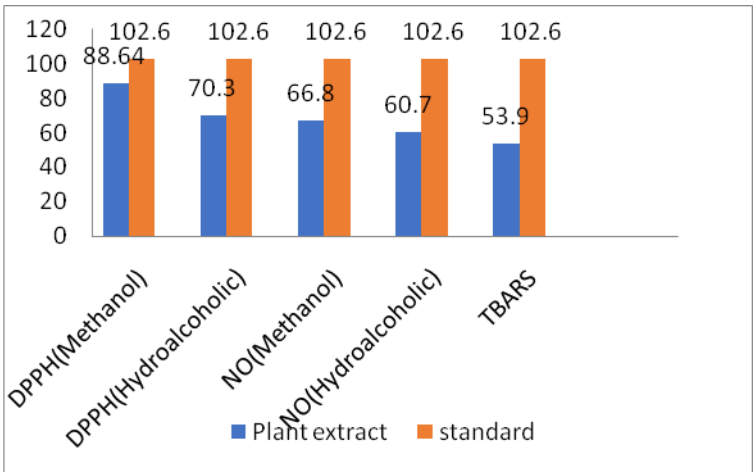

Fig 6: IC_{50} value of plant extract (*Tridax procumbens*) and standard (Ascorbic acid) in DPPH by methanolic and Hydroalcoholic solution, Nitric oxide (NO) scavenging assay of Methanolic and Hydroalcoholic solution and TBARS assay.

In the above graph shows, there is significant increase in the Absorbance on the increase in the Concentration for Soxhlet extraction by methanolic solution as compared to the Simple maceration with Hydro-alcoholic extraction.

Plant extracts were found to scavenge the nitric oxide with increasing the concentration.

Anti-lipid peroxidation activity of extracts was found to be increased with increasing the concentration.

Table 4: Methanolic flower extracts by TBARS assay method

S. No.	Test Compounds	IC50 values ± SEM (µg/ml)
		TBARS assay
1	Methanolic flower extract	53.9±1.1

2	Ascorbic acid	102.6

DISCUSSION

Tridax procumbens leaves, roots and whole plants have already proved for its anticoagulant, anti-leishmanial, anti-cancer, immunomodulatory agent, insecticidal, anthelmintic cardiovascular, antiseptic, antimicrobial, and insecticidal properties. *Tridax procumbens* have been used from ancient times to treat wounds, skin diseases and to stop blood clotting in folk medicine. Phytochemical studies showed that the*T. procumbens* is a rich source of flavonoids with a percentage of flavones and flavanones most commonly present in the Asteraceae family. In addition, flavonoids play an important role to control the growth of toxin-producing bacteria in plants. Flavonoids like Luteolin, glucoluteolin, quercetin and isoquercetin may also be attributed to antioxidant and antihyperlipidemic activity for other plant extracts have been reported.

A high-cholesterol diet regarded as an important factor in the development of cardiac diseases, since it leads to the development of hyperlipidemia, atherosclerosis, and ischemic heart disease. A variety of mechanisms, i.e. inhibition of mevalonate pathway, which inhibit HMG CoA reductase enzyme, decrease in NO bioavailability and cGMP metabolism, increase in free radical and peroxynitrite formation have been shown to play a role in cardiac effects of hyperlipidemia.

Recent studies have demonstrated that increased formation of free radicals or reactive oxygen species (ROS) contribute to cardiovascular disease progression. The generation of large amounts of reactive oxygen species can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification and DNA breaks. ROS induced depletion of antioxidants is a key factor for initiation of atherosclerosis.

DPPH is a free radical, which produces a violet solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncolored methanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidants. Methanolic flower extract of *Tridax procumbens* and hydro alcoholic flower extract has showed highest free radical scavenging activity against DPPH and NO free radicals. In that, Methanolic flower extract showed more activity than hydroalcoholic solution. It is strongly suggested that DPPH free radical abstracts the phenolic hydrogen of the electron – donating molecule and this could be the general mechanism of the scavenging action of anti-peroxidative flavonols.

The anti hyperlipidemic activity may be attributed to the inhibition of ROS by the flavonoids and triterpenoids present in the extracts. All the beneficial effects of the extracts may be due to their antioxidant and anti hyperlipidemic effects carried out by flavonoids, saponins and triterpenoids present in them.

CONCLUSION

This study evaluated antioxidant and antihyperlipidaemic activity of flower extracts of *Tridax procumbens Linn.* Phytochemical screening exhibits the presence of alkaloids, tannins, saponins, triterpenoids and flavonoids. Presence of phenolic compounds evidenced with TLC. Also antioxidant activity and antihyperlipidaemic activity was evaluated by DPPH invitro assay method and Lipid peroxidation method respectively. So this *Tridax procumbens linn* flower extracts exerts antioxidant and antihyperlipidaemic activity and thus needed to identify the bioactive compounds by usings further studies.

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