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In-vitro Antioxidant and Anti-inflammatory Activities of *Quisqualis indica* Linn. Leaves Extract

Pallavi Pal[1] and Ajeet Singh[1*]

[1]Department of Biotechnology, G.B. Pant Engineering College, Ghurdauri, Pauri, Uttarakhand, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author AS conceived and designed the experiment. Author PP performed the experiment. Authors AS and PP analyzed the data and wrote the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: In this study antioxidant and anti-inflammatory effect of ethanolic extract *of Quisqualis indica* leaves was evaluated.
Study Design: *In-vitro* analysis of Q*uisqualis indica* leaf extract.
Place and Duration of Study: Molecular Biology laboratory, Department of Biotechnology, G.B Pant Engineering College, Pauri, between July 2015 and July 2016.
Methods: Non-enzymatic and enzymatic assays such as DPPH (1, 1diphenyl-2-picryl hydrazyl), FRAP assay, superoxide dismutase SOD (EC 1.15.1.1), catalase (EC 1.11.1.6), for radical scavenging activity of ethanolic extracts of *Quisqualis indica Linn.* plant leaves had done. For estimation of anti-inflammatory action, two methods were employed: protein denaturation method and membrane stabilization method.
Results: Ethanolic extract of leaves on higher concentration had better antioxidant potential when compared with reference standard ascorbic acid. They exhibited strong antioxidant radical scavenging activity values for ethanolic extract of leaves. Results of anti-inflammatory method suggested better potential values for ethanolic extract and compared with standard drug diclofenac sodium respectively. A significant relationship between antioxidant, anti-inflammatory capacity and

**Corresponding author: E-mail: ajeetsoniyal@gmail.com;*

total phenolic content was examined, indicating that phenolic compounds are the major contributors for the antioxidant and anti-inflammatory properties of this plant.
Conclusion: Ethanolic extract of *Q. indica* exhibited strong anti-inflammatory and antioxidant activity and this can be used for designing novel drug inhibitors with better efficacy.

Keywords: Quisqualis indica Linn.; anti-oxidant; anti-inflammatory; DPPH (1, 1diphenyl-2-picryl hydrazyl; FRAP (Ferric reducing antioxidant power); SOD (superoxide dismutase); Catalase.

1. INTRODUCTION

Vegetations and their products fortreatment of diseases have been used extensively by humans for several years [1]. Medicinal plants are the richest bio-resources of folk's medicines and ancient systems of medicine; and food supplements, nutraceuticals, pharmaceuticals [2]. Different types of extracts of medicinal plants were used in the treatment of many health issues like microorganisms and polygenic disorders, cancer and infectious diseases. Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defense system. Free radicals are capable of reacting with, nucleic acids, proteins, membrane lipids and enzymes and other small molecules, which results in cellular injury [3].

Phytochemicals isolated from plants behold a defense mechanism to shield against these harmful reactive oxygen species (ROIs). The defense mechanism involved in antioxidant and intracellular enzymes like superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidase (GPX), catalase (CAT), and ascorbate Peroxidase (APX). The non-enzymatic antioxidants like glutathione, ascorbic acid are recently used [4].

Inflammation, which is a functionally defensive response, can be considered as a complex series of events that develop once the body got injured either by mechanical or chemical agents. Synthetic antioxidants and non-steroidal anti-inflammatory drugs (NSAIDs) are commercially accessible and presently used. However, these chemicals are harmful and their risk to health has exaggerated the demand for natural antioxidants [5].

Anti-inflammatory action is considered to be inhibition of PG (prostaglandin) synthesis particularly significantly it inhibits the COX (Cyclooxygenase) at the location of injury, as the decrease the prostaglandin E2 and prostacyclin reduces dilation and indirectly, oedema [6]. Inflammation plays a vital role within the promotional stage of carcinogenesis. Early within the inflammatory response, pro-inflammatory mediators reminiscent of prostaglandins and leukotrienes play a crucial role [7]. They have been associated with pathophysiology of certain types of human cancers as well as inflammatory disorders. Continuous production of these molecules in chronic inflammation has been linked to the development of cancer [8].

The human body system consists of several mechanisms which are capable to work against oxidative stress by producing antioxidants, which are created either naturally, or provided externally through foods and/or supplements. Free radical scavengers act as antioxidants by rebuilding and protecting damages caused by ROS (Reactive oxygen species) and RNS (Reactive nitrogen species), and as a result, lower down the risk of cancer and chronic diseases and will improve the immune system [9-13].

Quisqualis indica Linn. showing various medicinal activities such as anti-inflammatory drug activity, antipyretic activity, immune-modulatory activity, anti-staphylococcal activity, anthelmintic activity, antiseptic activity etc due to the presence of assorted active constituents all over the parts. This plant contains some medicinally active phytochemical constituents which are accountable for numerous medicinal activities [14].

Decoctions of the root, seed or fruit can be used as anthelmintic to expel parasitic worms or for alleviating diarrhea. Leaves and roots extracts are anthelmintic. Juice of leaves relieve flatulence. Infusion of leaves is used externally to treat boils and ulcers. Seeds are anthelmintic given to children to expel the worms [15,16].

Leaves consist rutin, trigonelline, L-proline, asparagine and quisqualic acid whereas flower gum contains pelargonidin-3-glucoside. Seed Oil contains linoleic, oleic, palmitic, stearic and

arachidic acids. Ellagitannins, quisqualin A and quisqualin B is present in fruits of this plant and flower carries linalool oxides (furanoid and pyranoid), 2, 2, 6-trimethyl- 6- vinyl-3-oxo tetrahydropyran, (E, E)-alphafarnesene, (Z)-3-hexenyl benzoate and benzyl benzoate [17]. The aim of the present study is to analyze the ethanolic extract of *Quisqualis indica* leaves. Different assays were employed for the assessment of antioxidant and anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1 Plant Material and Sample Preparation

The leaves of *Quisqualis indica* Linn was collected from Kotdwar region, Uttarakhand. Plant samples were dried at room temperature and store for future use. Dried leaves are now completely free of moisture and were finely ground by using grinder. Powdered dried leaves (10 g) were extracted with 250 ml of ethanol using soxhlet apparatus for 24 hrs. The extract was concentrated using vacuum rotary evaporator at 64℃. After cooling, the extract was filtered through Whatmann filter paper No.1 and then to get dry residue filtrate was evaporated through rotary evaporator and stored at 4℃ for further experimentation.

2.2 Chemicals and Reagents Used

Ethanol, DPPH (1, 1diphenyl-2-picryl hydrazyl), NBT (Nitro blue tetrazolium), NaOH (Sodium hydroxide), FC (Folin-Ciocalteu's reagent), $FeCl_3$ (Ferric chloride), Gallic acid, Ascorbic acid, Benedict's qualitative solution, Glucose, EDTA, $NaHCO_3$ (Sodium bi-carbonate), Tri-chloroacetic acid, HCL (Hydrochloric acid), H_2O_2 (Hydrogen peroxide), sodium citrate was purchased from HI-MEDIA (India).

2.3 Preliminary Phytochemical Screening

Phytochemical screening of ethanolic extract of leaves of *Quisqualis indica* Linn was done [18, 19].

2.3.1 Reducing sugar

1g of plant extract was added to 10 ml of distilled water and the mixture was boiled for 5 min. Then, mixture was filtered and made alkaline with 20% sodium hydroxide solution. The mixture was boiled with an equal volume of Benedict's qualitative solution in a water bath. The brick-red formation shows the presence of reducing sugar.

2.3.2 Saponins

2 g of plant extract was added to 20 ml of distilled water and boiled in water bath and then, filtered. 5 ml of distilled water was added to the filtrate and shaken for froth formation. The froth was mixed with some drops of oil and shaken vigorously again and the formation of emulsion revealed the presence of saponins.

2.3.3 Tannins

0.5 g of plant extract was added to 20 ml distilled water and boiled in water bath and filtered. Few drop of 0.1% ferric chloride was added to the filtrate. Formation of brownish green or blue black color indicated the presence of tannins.

2.3.4 Phenols

prepare 10% ferric chloride and 3-4 drops are added to 0.5 g of plant extract. Bluish black or dark green color depicted the presence of phenols.

2.3.5 Alkaloids

Prepare Wagner's reagent (1.27 g of iodine in 2 g of potassium iodide in 100 ml of water) and 3-4 drops was added to 0.5 g of plant extract. The formation of reddish brown color showed the presence of alkaloids.

2.3.6 Quinones

2 ml concentrated HCL was added to 0.5 g of plant extract and the formation of yellow color revealed the presence of quinines.

2.3.7 Flavonoids

10 ml ethyl acetate with 0.5 g of plant extract was heated over a steam bath for 5 min. The mixture was filtered and 4 ml filtrate mix with 1 ml of dilute ammonia solution. Yellow color indicated the presence of flavonoids.

2.3.8 Steroids

0.5 g of plant extract with 2 ml concentrated H_2SO_4 was added to 2 ml of acetic anhydride. The color appeared from violet to blue or green which indicated the presence of steroids.

2.3.9 Soluble starch

Prepare 5% KOH and mix 3- 4 drops with 1 g of plant extract and boiled. Mix few drops of H_2SO_4 into after cooling. Yellow coloration showed the presence of soluble starch.

3. QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC CONTENT

The concentration of phenolics in ethanolic leaf extract was determined using spectrophotometric method [20]. Ethanolic extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.5 ml ethanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The test sample was incubated at room temperature for 90 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicates for analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration curve was constructed. Based on the measured absorbance, the concentration of phenolics was estimated (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

4. ASSESSMENT OF *In-vitro* ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *Quisqualis indica* LEAVES

4.1 DPPH (1, 1diphenyl-2-picryl hydrazyl) Assay

The antioxidant activity of the extract was measured with DPPH methodology with slight modifications [21]. A solution of DPPH was freshly ready by dissolving 6 mg DPPH in 50 ml ethanol (0.3 Mm). The leaf extract with different concentrations (10, 15, 20, 25, 30 mg/ml) was prepared with ethanol. Freshly prepared DPPH solution in ethanol was added to each test tube with various concentrations. The reaction mixtures were allowed to incubate in dark for 30 min at room temperature. After 30 min. the absorbance was recorded at 517 nm using UV-Vis spectrophotometer against ethanol as blank. The percent inhibition of radicals was calculated using following formula,

$$\% \text{ inhibition} = [\text{O.D (control)} - \text{O.D (sample)}] / \text{O.D (control)}] \times 100$$

Where, absorbance (control) is the absorbance of DPPH solution without extract and Absorbance (sample) is the absorbance of sample with DPPH solution. The half maximal inhibitory concentration (IC50) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%. Ascorbic acid was used as standard. All the tests were performed in triplicates and the graph was plotted with mean values.

4.2 Ferric –reducing Antioxidant Power (FRAP) Assay

The procedure was used to determine reducing power of extract with the slight modifications [22]. This reducing power was investigated by observing the transformation of colorless ferric complex Fe^{3+} to a blue colored ferrous complex Fe^{2+} by the action of electron donating antioxidants. The reduction is monitored by measuring the change of absorbance at 700 nm. The working FRAP reagent was prepared daily by mixing 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml) in a test tube followed by incubating in a water bath at 50°C for 30 min. Then 1.25 ml of tri-chloroacetic acid (10%) was added to the mixture and for 10 min these tubes were centrifuged (13000 g, 10 min.) upper layer of solution was taken and diluted with distilled water (2.5 ml) and freshly prepared ferric chloride (2.5 ml, 0.1%). The reaction mixture was mixed thoroughly and absorbance was measured at 700 nm. Ascorbic acid was used as standard and phosphate buffer as blank.

$$\% \text{ inhibition} = [\text{O.D (control)} - \text{O.D (sample)}] / \text{O.D (control)}] \times 100$$

4.3 SOD (Superoxide Dismutase) Assay

This activity was determined by the method of NBT (nitro blue tetrazolium reagent) reduction with slight modifications [23]. In this, we measure the ability to inhibit this photochemical reagent in a reaction mixture consist 50 mM phosphate buffer, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA, and leaf extract with different concentrations. Blanks were not incubated under light whereas extract samples

were illuminated for 10 min under light lamp (40 W). The reaction mixture without enzyme extract developed maximum color at 560 nm. One enzyme unit has been quantified on the basis of % inhibition of NBT. The activity of SOD is expressed as units/g protein. The SOD activity was calculated by the following formula:

% inhibition of nitrite formation = [O.D (control) - O.D (sample)] / O.D (control)] x 100

4.4 Catalase (CAT) Assay

The method mentioned by Aebi H was used to measure the activity of catalase with slight modifications [24]. The enzyme extract (0.1 ml) was added to the reaction mixture containing 3 ml of H_2O_2 and 0.01M phosphate buffer (pH 7.0) and the O.D change was measured at 240 nm. The decomposition of H_2O_2 was followed as a decrease in absorbance at 240 nm in a UV-VIS spectrophotometer. The time taken for decrease in the absorbance from 0.45 to 0.4 is noted as ΔT. The activity of the enzyme is expressed in the terms of µM of H_2O_2 consumed/ min/ mg protein. The activity of catalase was calculated by the following formula,

Units in assay mixture = 17 / Δt

Where, Δt = time in minutes.

5. ASSESSMENT OF *In-vitro* ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *Quisqualis indica* LEAVES

5.1 Protein Denaturation Method

The anti-inflammatory activity of *Quisqualis indica* leaves extract was evaluated by inhibition of albumin denaturation with the slight modifications [25]. The reaction mixture (5 ml) consists of 2 ml of various concentration of leaves extract (100 mg/ml) and 2.8 ml of egg albumin (from fresh hen's egg). Then the reaction mixture was incubated at (27 ± 1)°C for 15 minutes and then heated at 70°C in a water bath for 10 minutes for inducing denaturation. Then after cooling, the absorbance was measured using UV-VIS spectrophotometer at 660 nm. Double distilled water used as blank. Diclofenac sodium, a non-steroidal anti-inflammatory drug was used as a standard drug. Experiment was done in duplicates and average was taken. The percentage of inhibition of protein denaturation was calculated as follows given below-

% inhibition = [O.D (control) – O.D (sample)] / O.D (control)] x 100

5.2 Membrane Stabilization Method

The anti-inflammatory activity of *Quisqualis indica Linn* leaves extract was determined by using RBC membrane stabilization method with slight modifications [26]. Blood Was collected from healthy volunteer and mixed with equal amount of Alsever's solution (2% glucose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride in water) and centrifuged at 3000 rpm for 10 minutes. The packed cells were washed with iso-saline solution (0.9%) and a 10% solution was made by using isosaline solution.

Different concentration of extract and reference drug were prepared and mixed with the reaction mixture consisting hypo saline solution (2ml), phosphate buffer (1 ml), 0.36% RBC suspension (0.5 ml) into each test tube. All tubes were kept for incubation at 37°C for 30 minutes in a BOD incubator. After this, solutions were centrifuged at 3000 rpm.

Hemoglobin content was measured by using supernatant liquid spectrophotometricaly at 560 nm. The membrane stabilization or inhibition was estimated by using the formula given below-

% inhibition = [O.D (control) – O.D (sample)] / O.D (control)] x 100

5.3 Statistics Analysis

All analyses were done at least in triplicates and these values were then expressed as average values along with their standard deviation. Data were expressed as means standard errors. Differences were analyzed by ANOVA. All data were presented as mean values and standard mean errors for the replicate sets of experiments in each case.

6. RESULTS AND DISCUSSION

6.1 Screening of Phytochemicals

Phenolic are grouping of highly hydroxylated compounds present in extractive fractions of several plant parts such as stem, flower, leaves, bark etc. In the present study, tannins, reducing sugar, saponins, flavonoids and alkaloids were

present in the leaf extract of *Quisqualis indica Linn.*

Table 1. Phytochemical analysis of ethanolic extract of *Quisqualis indica* Linn leaves

S. no.	Phytochemicals	Screening of ethanolic leaf extract + = Present - =Absent
1.	Tannins	+
2.	Steroids	_
3.	Reducing Sugar	+
4.	Saponins	+
5.	Soluble starch	_
6.	Flavonoids	+
7.	Alkaloids	+
8.	Quinones	_

6.2 The Content of Total Phenolics

The total phenolic content (TPC) of ethanol extracts of *Quisqualis indica* was determined by Folin– Ciocalteu assay. According to literature, ethanolic extracts show the higher content of phenolic compounds than other extracts; phenols are also the main compounds which are responsible for the antioxidant activity; for this reason, ethanolic extracts of leaves have been further analyzed [21]. Results of ethanolic extracts were expressed as mg of Gallic acid equivalent (GAE)/g of extract Table 2, by using a standard curve of gallic acid. *Quisqualis indica* leaves displayed quantitative in TPC, with a mean value of 25.47 mg GAE/g. The TPC ranged from 5.49 ± 1.03 to 49.07 ± 4.87 mg GAE/g of dried extract.

Table 2.Total phenolic content of *Quisquails indica* Linn. leaves expressed in terms of GAE

S. no.	Concentration (mg/ml)	Total Phenol content (mg Gallic acid /g equivalent)
1.	10	5
2.	20	17
3.	30	22
4.	40	36
5.	50	49

Polyphenols play important role in adsorbing and neutralizing free radical, quenching oxygen this is mainly due to their redox properties. Assays measure the capacity of an antioxidant to reduce an oxidant, which changes the color when reduced. The degree of color change is directly related to the sample's antioxidant concentration. Hydrogen peroxide scavenging activity especially of phenol compounds which are assigned for their electron-donating ability. Phenolic and flavonoids are the poly-phenolic compounds which have been found to have free radical scavenging activity [27].

Phenolic compounds donate hydrogen molecules to free radicals and thus act as antioxidants. In this study, we determined the free radical scavenging capacity of the leaf extract using DPPH, and their ferric reducing capacities using the FRAP assay. DPPH and FRAP assays have been widely used to calculate the antioxidant capacity of plant extract.

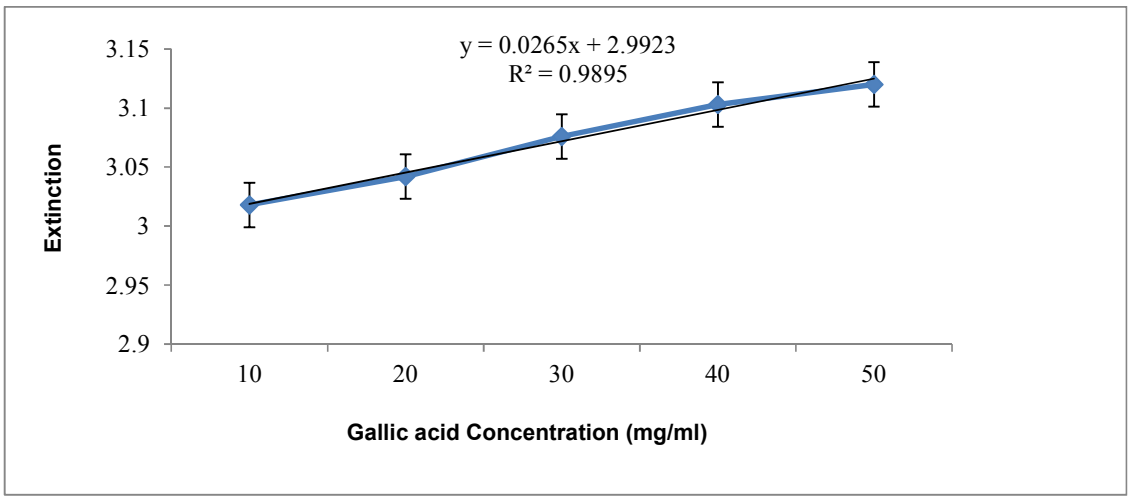

Fig. 1. Calibration curve for standard Gallic acid

6.3 Assessment of *In-vitro* Antioxidant Activity of Ethanolic Extract of *Quisqualis indica* Linn. Leaves

Antioxidant activity of *Quisqualis indica* extracts obtained with ethanol solvent was investigated by DPPH method, Because DPPH a strong absorption band is centered at about 515 nm, the solution of DPPH radical form in deep violet in colour and it becomes colorless to pale yellow when reduced upon reaction with hydrogen donor [28]. The decrease in absorbance depends linearly on antioxidant concentration. Ascorbic acid is used as standard antioxidant and obtained results are presented in (Table. 2). Results are expressed as percent of DPPH inhibition (I%) and ascorbic acid (AA) equivalents g-1.

6.3.1 Free-radical scavenging effect

During aerobic metabolism as well as in the process of drug bio-transformation, reactive oxygen species (ROS) are produced as by-products. These radicals include such as superoxide anion (O^-), hydroxyl radical (HO), alkoxyl radical (RO), peroxyl radical (ROO) and non-radicals such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) [27].

Free radicals can cause lipid oxidation, protein oxidation, DNA strand breaks, and modulation of gene expression. These Reactive oxygen species are involved in liver diseases and also lead to atherosclerosis, cancer, stroke, asthma, arthritis and other age-related diseases [28].

To evaluate the scavenging effect of DPPH in ethanolic fraction, enzymatic and non- enzymatic radical scavenging assays were performed and the results were compared with ascorbic acid as shown in Fig. 2. The scavenging ability of ethanolic extract of leaves of *Q. Indica* was concentration-dependent and expressed as the values of % inhibition. At higher concentration, 71.609± 0.029 and 65.282±0.032 for ascorbic acid and ethanolic extract were estimated and exhibit IC_{50} values 2.43 and 3.28 (mg/ml) respectively in compare to previously reported study [19]. It means ethanolic extract of plant leaves at higher concentration captured more free radicals formed by DPPH resulting into a decrease in absorbance and increase in IC_{50} value.

6.3.2 Reducing power of leaf extract and ascorbic acid

The reducing power of the extract, which may serve as a reflection of its antioxidant activity, was determined using a modified Fe^{3+} to Fe^{2+} reduction assay, whereby the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the sample.

Reducing power assay is associated with antioxidant activity and may serve as a significant reflection of the antioxidant effect.

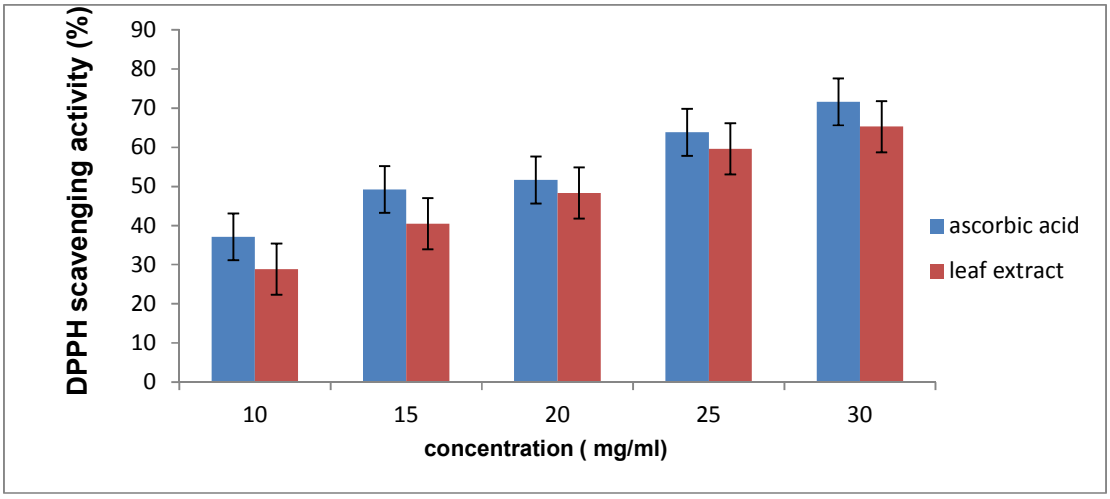

Fig. 2. DPPH free radical scavenging activity by ethanolic leaf extract of *Q. indica*. and ascorbic acid

Values are mean of three replicate determinations (n = 3) ± standard deviation

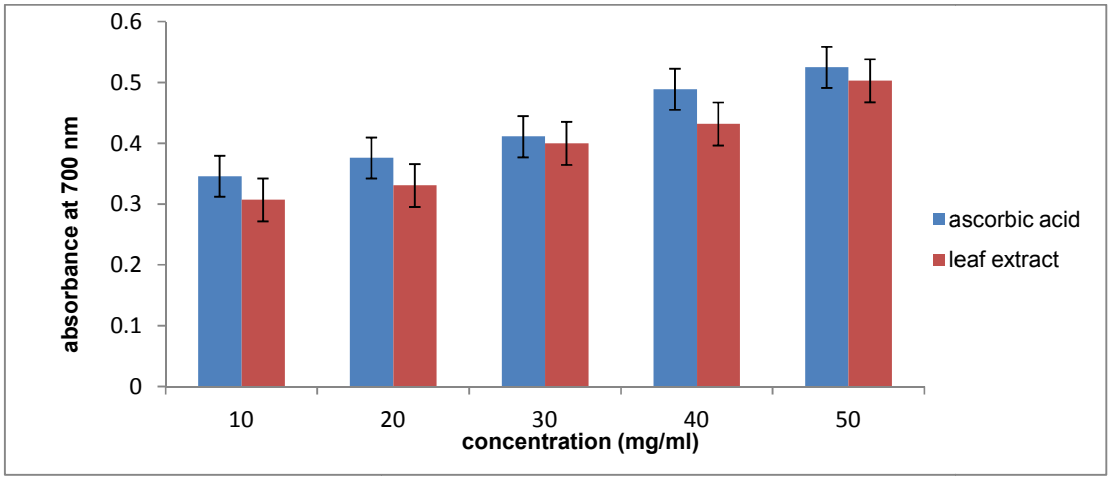

Fig. 3. Reducing power of ethanolic leaf extract of *Q. indica* and ascorbic acid (reference drug) respectively

Values are mean of three replicate determinations (n = 3) ± standard deviation

Phytochemical compounds possess reducing power indicates that they are electron donors and can also reduce the intermediates of lipid peroxidation processes which are being oxidized so that they can act as primary and secondary antioxidants [29].

For the measurements of the reducing ability, the Fe3+ – Fe2+ transformation was investigated. The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity [30]. (Fig. 3) showed values 0.525±0.24 and 0.503±0.19 for ascorbic acid and ethanolic leaves extract respectively. The reducing power of the extracts improved with an increase in concentration.

6.3.3 Superoxide anion scavenging (SOD) effect

SOD action responsible for the formation of H_2O_2, a highly toxic molecule which needs to be eliminated. In cellular oxidation reactions O_2^- radicals are formed when oxygen is reduced. Superoxide radicals are inactivated by the enzyme superoxide dismutase (SOD), the only enzyme known to use a free radical as a substrate.

SOD generates H_2O_2 as a product which is in turn more toxic to the cells and requires catalase or peroxidases to scavenge. Thus a concomitant increase in catalase and or Peroxidase is

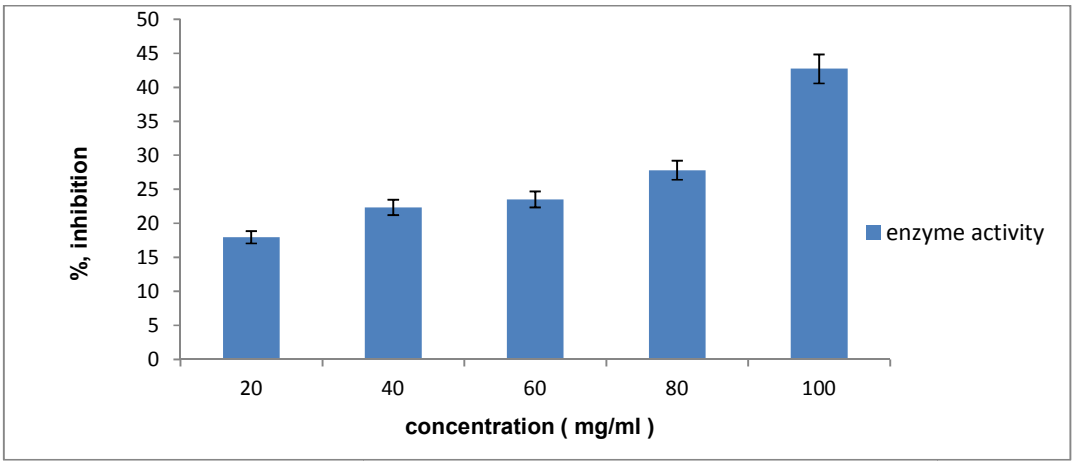

Fig. 4. Percent inhibition of super oxide radical scavenging effects by ethanolic *Q. indica* leaves extract

Values are mean of three replicate determinations (n = 3) ± standard deviation

essential for the beneficial effect from increase in Superoxide dismutase activity [31].

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). As in (Fig. 4) value 42.721± 0.40 shown for (SOD) activity against super oxide radicals. Activity of ethanolic leaf extract of *Quisqualis indica* increased with increased concentrations.

6.3.4 Catalase effect

Catalase is a tetrahedral protein, produced by the body to break down hydrogen peroxide (H_2O_2) into water and oxygen gas. H_2O_2 is a by-product of respiration and cells make the enzyme catalase to remove hydrogen peroxide. This plays a significant role in scavenging H_2O_2 mainly in chloroplast and in maintaining redox status of cell. Hydrogen peroxide, a reactive non-radical compound, is very important as it can penetrate biological membranes and causes damages to the cell body [32].

but it can generate the highly reactive hydroxyl radical (HO), it may convert into species which are more reactive such as singlet oxygen and hydroxyl radicals, through the Fenton reaction and is found to be main reason for toxicity associated with hydrogen peroxide [33].

Hydrogen peroxide can be formed by several oxidizing enzymes *in-vivo* such as superoxide dismutase. It can permeate through biological membranes and slowly oxidizing number of compounds. Hydrogen peroxide is used in the respiratory burst of activated phagocytes [34]. Hydrogen peroxide can de-activate enzymes involved in cellular energy production such as glyceraldehyde-3-phosphate dehydrogenase found in glycolytic pathway [35]. In this study, values are shown in (Fig. 5), at highest concentration 30 (mg/ml) value of enzyme activity 2.187±0.049 unit/litre shown. Catalase activity for ethanolic leaf extract increased with concentrations.

6.4 *In-vitro* Anti-inflammatory of *Q. indica* Leaf Extract

6.4.1 Protein denaturation method

Denaturation of protein is a well-documented cause of rheumatoid arthritis. The mechanism of denaturation involves alteration of hydrophobic, electrostatic hydrogen, and disulfide bonding [36].

Membrane stabilizing activity was done on erythrocyte membrane. Erythrocyte membrane is analogous to lysosomal membranes. The lysosomal enzymes released during inflammation produce variety of disorders. This test was taken as a measure of anti-inflammatory activity and results showed that ethanolic extract protects erythrocyte membrane. The activity compared to that of diclofenac. Chemical evaluation of the ethanolic extract of leaves indicates that flavonoids, terpenoids, phenol and tannins are present in leaves extract. These phytochemicals are responsible in preventing the free radical attack and capable of stabilizing the lysosomal membrane there by preventing inflammatory process [37]. This Study exhibited % inhibition values for the prevention of protein denaturation at higher concentration (50 mg /ml) was shown 76.923±0.28 and 71.031±0.35 for diclofenac sodium and ethanolic leaves extract respectively (Fig.6). IC_{50} value found to be as 3.04 and 2.71 (mg/ml) for ethanolic leaf extract and diclofenac sodium respectively. The activity was compared to that of diclofenac.

6.4.2 Membrane stabilization method

The lysosomal membrane was thought for an extended time to primarily act as a physical barrier separating the luminal acidic environment from the cytoplasmic environment. Meanwhile, it's been accomplished that distinctive lysosomal membranes play essential roles during a variety of cellular events ranging from phagocytosis, autophagy, cell death, virus infection to membrane repair [38]. At the onset of an inflammation, the cells bear activation and unharness inflammatory mediators that cause vasodilatation and exaggerated porousness of blood vessels resulting in the exudation of plasma proteins and fluids into the tissues [39].

This stabilization of lysosomal membrane is important in limiting the inflammatory response by restricting the release of lysosomal constituents which are released by the action of hydrolytic enzymes [40]. From the present study it can be stated that the extract of the leaves of *Quisqualis indica* is capable of controlling the production of auto antigens due to denaturation of proteins and stabilize the lysosomal membranes *in-vivo.*

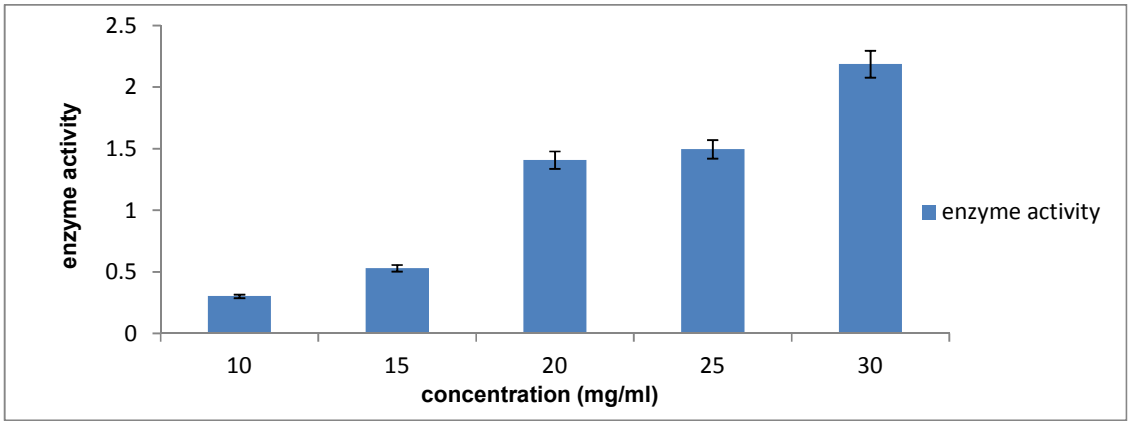

Fig. 5. Catalase activity by ethanolic extract of *Q. indica* leaves
Values are mean of three replicate determinations (n = 3) ± standard deviation

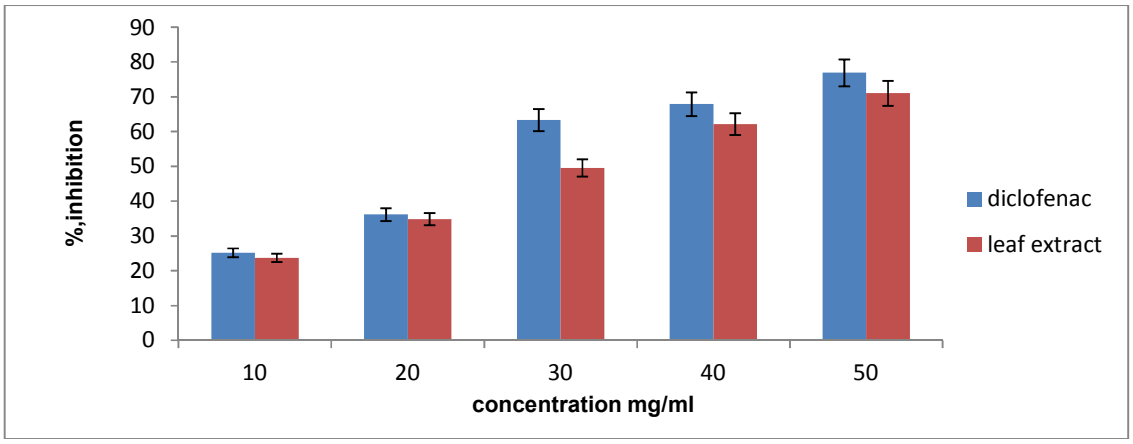

Fig. 6. Percent inhibition of protein denaturation by ethanolic leaf extract of *Q. indica* Linn *and* diclofenac sodium (drug)
Values are mean of three replicate determinations (n = 3) ± standard deviation

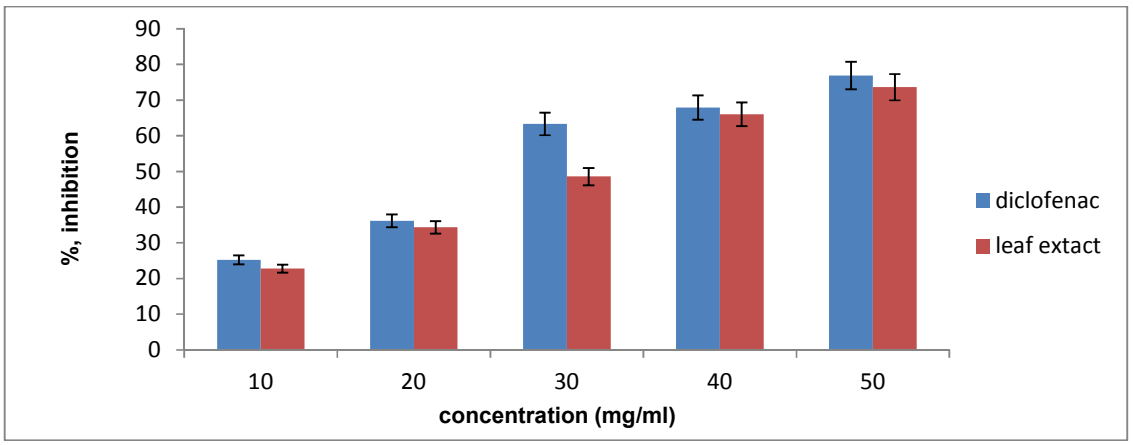

Fig. 7. Percent inhibition of membrane stability by ethanolic leaf extract of *Q. indica* Linn and diclofenac sodium (drug)
Values are mean of three replicate determinations (n = 3) ± standard deviation

Results of membrane stabilization method is showed in (Fig. 7) %, Percent Inhibition values for the prevention of membrane is shown 76.923±0.028 and 73.624±0.024 for diclofenac sodium and ethanolic extract respectively. Nevertheless, the IC_{50} values were 3.14 and 2.71 (mg/ml) for ethanolic extract and diclofenac sodium respectively in compare to previously reported study, [16]. The activity was compared to that of diclofenac.

7. CONCLUSION

In the present study, results indicate that the ethanolic extract of *Quisqualis indica* leave possesses strong *in-vitro* anti-inflammatory and antioxidant properties. The result of present study supports partially the use of plant leaves as better antioxidant and anti-inflammatory drug. This investigation further supports the view that leaves of this plant are promising sources of natural antioxidants as well as for anti-inflammatory effect. A significant correlation between antioxidant properties and total phenolic content was found, indicating that phenolic compounds are the major contributor to the antioxidant properties of plant leaf extract. Furthermore, the *in-vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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