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Estimation of total phenol and total flavanoid content with Antioxidant and anti-inflammatory activity of *Bambusa arundinacae* (bamboo shoot) grown in Nilgiris

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ABSTRACT

This work reports the determination of the total contents of flavonoids and phenolic acids of two extracts of the bamboo shoots and on this basis, studied the free-radical scavenging function of the ethanol and hydro alcoholic extracts by the DPPH, total antioxidant capacity, Hydrogen peroxide, superoxide and Lipid peroxide method and anti-inflammatory activity by the protein denaturation and HRBC membrane stabilization method. The experimental results show that there are large amounts of flavonoids and phenolic acids in the bamboo shoots. Through the research of antioxidant and anti-inflammatory activity it shows that the extracts of the bamboo shoots have strong free-radical scavenging power. By doing the relevant analysis for the contents and antioxidant and anti-inflammatory potential of the bamboo shoot extracts, we know that there is strong correlation between the contents of phenols and flavonoids in the bamboo shoots. It is concluded that the hydro alcoholic extract of bamboo shoot have strong antioxidant and anti-inflammatory activity, thus provide scientific data for their further development and exploitation.

Key words: DPPH, superoxide, anti-inflammatory, HRBC, antioxidant, free-radical etc.

INTRODUCTION

In earlier period, the free radicals have aroused extensive questioning among scientists. In human body Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are natural by products produced by endogenous metabolic route and exogenous chemicals. They are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), nitric Oxide (NO^-). Free radicals may be due to specific metabolic functions in the body or either by the accidents of chemistry[1]. Free radicals could be each oxygen derived or nitrogen derived[2].Our body has free radical scavenging antioxidants present within, which are derived from dietary sources like teas, vegetables and fruits[3]. ROS are also dependable in progress of systemic inflammatory effect and then they trigger nuclear factors

which induce the synthesis of cytokines. Later, adhesion molecules and inflammatory mediators are also formed. At the site of inflammation, free radicals react with different cell components thus leading to loss of function and cell death[4]. Inflammation is a complex process is associated with pain and involves occurrences such as, the increase of vascular permeability, increase of protein denaturation and membrane alteration[5].

Since time immemorial many herbal plants have been prove to have therapeutically potential[6]. Bamboo as an herbal drug is used in various traditional system of medicine like ayurveda[7]. It is about 90 genera and 1200 species of bamboo found in the world and plays an important economic role[8]. Bamboo a reliable woody unrefined material related to urban and rural india[9]. The bamboo shoot is considered as one of the widely applauded nutrient rich food items due to the presence of high content of protein, amino acids, minerals, fibre, carbohydrates, and low fat[10]. The major phytoconstituents are triterpines and steroid glycosides which are responsible for the pharmacological activity[11]. Bamboo contain flavanoids which have antioxidant and reduce inflammation by promoting blood circulation and inhibit allergic reactions. The antihypertensive, antitumour and antihyperlipidemic properties of the bamboo shoot have also been proven to possess cholesterol-lowering activity[12].The beneficial therapeutic effect of these medicinal herbs is seen in their continued use and proven scientifically[13]. Bamboo is called as Green gold and nowadays people are switching over the use of herbal medicine instead of the manmade drugs and have capacious nutraceutical properties and thus form a core feature for research in many laboratory[14]. Herbal plants contain a blend of phytochemical such as phenols, flavonoids, terpenoids, vitamins which acquire antioxidant activity. These phytochemicals and natural antioxidants reveal an extensive favourable organic outcome and could counterbalance oxidation of biological molecules by scavenging free radicals and chelating free catalytic metals[1]. Thus, the main aim of this study is to estimate the phenolic and flavanoid content and evaluate the antioxidant and anti-inflammatiry activities of the two different extracts of bamboo shoot extracted by two different solvents (ethanol and hydro alcohol).

MATERIALS AND METHODS

Collection, Authentication: The young bamboo shoot was collected from the forest near Doddabetta, Nilgiris, Tamilnadu and was authenticated by Dr. S. Rajan, Field Taxonist'Survey of Medicinal plant and Collection, Department of Ayush, Emerald, the Nilgiris. The materials used for the study are fresh bamboo shoots that are commonly used as raw food and medicine.

Chemicals and drugs: 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), ferric chloride, sodiumphosphate Phosphate buffer saline, Ammonium molybdate (4mm),sodium sulphate (28mm), Egg lecithin, sulphuric acid, phosphate buffer (7.4) ascorbic acid (200mm), Ferric chloride (400mm),Trichloroacetic acid15%w/v,Tributanol acetic acid (0.375%w/v), Trypsin, Dimethyl sulphoxide, Trypsin, Rutin Gallic acid, Aspirin, Diclofenac sodium. All the chemicals were purchased from Sigma Aldrich.

Methodology for extraction: The method used to dry bamboo shoots is shade drying. Deriving extracts from bamboo shoots was done by the cold maceration followed by successive extraction method using ethanol and hydro alcohol. At the end of each respective extraction; the extracts were filtered using Whatma1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator (Buchi,Switzerland).The percentage yield of extracts was calculated. The extracts were used for the preliminary screening of phytochemicals.

Qualitative phytochemical screening: A small amount of the dry extract was used for the phytochemical tests for; phenols, flavonoids, alkaloids, glycosides, steroids, tannins, carbohydrates and proteins according to the standard method.

Total phenolic content[16]: The total phenolic content of two extracts was measured using colorimetric Folin-Ciocalteu method. The reaction mixture consisted 5 ml of diluted sample to which 3 ml of distilled water and 0.5 ml Folin-Ciocalteu reagent was added. After 3 minutes, add 2 ml of 20 % Na_2CO_3 solution and place the tubes in boiling water bath for one min, cooled and the absorbance was measured at 760 nm. Standard graph was prepared by using different concentration of Gallic acid.

Total flavonoid content[16]: The flavonoid content of different extracts was measured using an Aluminium chloride colorimetric method. 0.5 ml of sample was mixed with 0.5 ml of 2% $AlCl_3$ and incubated for 10 mins and the absorbance was measured at 415 nm. The measurement was compared to a standard graph rutin.

Evaluation of *in vitro* antioxidant activity

Total antioxidant capacity[17]: To 0.1 ml of the extract, 1ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate combined in eppendorf tube) was added .The tubes were capped and incubated at 350 C for 90 min. After cooling to room temperature the absorbance was measured at 695 nm against blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of Ascorbic acid.

DPPH radical quenching activity[18]: The DPPH assay was carried out according to procedures of Brand-William et al, with minor modifications. Different volumes (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µl) of ethanol and hydro alcoholic extracts were mixed with DPPH radical in methanol (2.2 mg/l, 200 µl) in a 96-well micro plate. The final volume of each well was made up to 300 µl by adding the appropriate amount of methanol. The absorbance was measured at 515 nm. The DPPH concentration in the reaction medium was calculated from a calibration curve derived from serial dilution of the DPPH standard. The control (containing all reagents except the test compound) and standards were subject to the same procedure. Ascorbic acid was used as the standard. The free radical scavenging activity was expressed as the percentage inhibition of free radical generation by the sample, and calculated using the following formula:

$$\% \textbf{ of inhibition} = \frac{(\textbf{ABS Control} - \textbf{ABS Sample})}{\textbf{ABS Control}} \times \textbf{100}$$

Where: ABS control is the absorbance of the control and ABS sample is the absorbance of the extract

Alkaline DMSO radical scavenging activity [19]: To 0.5 ml of various concentration of the extracts, 1 ml alkaline DMSO and 0.2 ml NBT 20mM (50 mg in 10ml phosphate buffer pH 7.4) were added. Different concentrations (25-500 g/ml) of the test solutions were added and absorbance was recorded at 560 nm against the control. Ascorbic acid was taken as standard

$$\% \textbf{ of inhibition} = \frac{(\textbf{ABS Control} - \textbf{ABS Sample})}{\textbf{ABS Control}} \times \textbf{100}$$

Where: ABS control is the absorbance of the control and ABS sample is the absorbance of the extract.

H_2O_2 radical scavenging activity[20]: Hydrogen peroxide scavenging activity of the extract was determined according to the method given by Ruch et al (1989). Hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). Extracts (1–0 µg/ml) were added to hydrogen peroxide solution (0.6 ml). After 10 min the absorbance of hydrogen peroxide was determined at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the reference compound.

$$\% \textbf{ of inhibition} = \frac{(\textbf{ABS Control} - \textbf{ABS Sample})}{\textbf{ABS Control}} \times \textbf{100}$$

Where: ABS control is the absorbance of the control and ABS sample is the absorbance of the extract

Lipid peroxidation assay[21]: The mixture (Egg phosphatidylcholine in 5 ml saline) was sonicated to get a homogeneous suspension of liposome. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid to a mixture containing liposome (0.1 ml). The pink chromogen was extracted with a constant volume of n-butanol and absorbance of the upper organic layer was measured at532 nm. The experiment was performed in triplicate

$$\% \textbf{ of inhibition} = \frac{(\textbf{ABS Control} - \textbf{ABS Sample})}{\textbf{ABS Control}} \times \textbf{100}$$

Evaluation of in vitro anti inflammatory activity

Protein denaturation method[21]: The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of ethanol and hydro alcoholic extracts of *Bambusa arundinacae*, so that final concentrations become(1000, 500, 250, 125, 62.5, 31.25µg/ml). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) ºC in a BOD incubator (Labline Technologies) for 15 min and then heated at 70ºC for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Ibuprofen at the final concentration of (1000, 500, 250, 125, 62.5, 31.25µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\textbf{\% inhibition} = \textbf{100} \times \textbf{(At / Ac - 1)}$$

Where: At = absorbance of test sample,
Ac = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

HRBC membrane stabilization method[22]: HRBC membrane stabilization method has been used to study the anti-inflammatory activity. Blood was collected from the healthy volunteers and mixed with sodium citrate. The blood was centrifuged at 3000rpm and packed cells were washed with isotonic saline (0.85%, pH 7.2). The assay mixture contains 50µl phosphate buffer [pH 7.4, 0.15 M], 100µl hypo saline [0.36 %], 25µl HRBC suspension[10 % v/v]) with 25µl of plant extracts of various concentrations (1000, 500, 250, 125, 62.5, 31.25µg/ml), standard drug Diclofenac sodium (1000, 500, 250, 125, 62.5, 31.25µg/ml) and control [distilled water instead of hypo saline to produce 100 % hemolysis] were incubated at 37º C for 30 min. The hemoglobin content in the suspension was estimated using Multimode Micro-plate Reader at 560 nm. The percentage hemolysis produced in the presence of distilled water was taken as 100 %. Percentage of HRBC membrane stabilization or protection was calculated using the formula

$$\textbf{\% Stabilization} = \textbf{100} - \textbf{[(Optical Density of Drug)} \div \textbf{(Optical Density of Control)} \times \textbf{100]}$$

Satistical analyses: The results were presented as mean±SD.All analyses were carried out in triplicates. Statistical data were performed by one way analysis of variance. Significant differences between groups were determined at $P<0.05$. Microsoft excel 2007 were used for the graphical and statistical evaluations.

RESULTS

Preliminary Phytochemical screening: Our preliminary phytochemical screening for shoots of *Bambusa arundinacae* (table 1) revealed the presence of phenols, carbohydrates, glycosides, steroids, flavonoids, proteins and tannins while alkaloids and tannins were absent**.**

Total phenols and flavonoids: The total phenolic content of ethanol and hydro alcoholic extract of *Bambusa arundinacae* was measured by Foline Ciocalteau reagent in terms of gallic acid equivalent was 34.6mg/ml and 78.48 mg/ml. The flavonoid content of the plant sample calculated as rutin equivalent was 81.44mg/and 108.48 mg/ml. The result shows that the phenolic and flavonoid content in the hydro alcoholic extract is more when compared to the ethanolic extract. It has been acknowledged that flavonoids show antioxidant activity and their property on human nutrition and health are significant. The outcome strongly recommends that the phenolics are chief

components of this plant, and some of the pharmacological result could be recognized to the presence of this invaluable component.

Table (1) Preliminary phytochemical screening

Phytoconsituents	Ethanolic Extract	Hydroalcoholic Extract
Phenols	+	+
Flavanoids	+	+
Alkaloids	-	-
Glycosides	+	+
Steroids	+	+
Tannins	-	+
Carbohydrates	+	+
Proteins	+	+

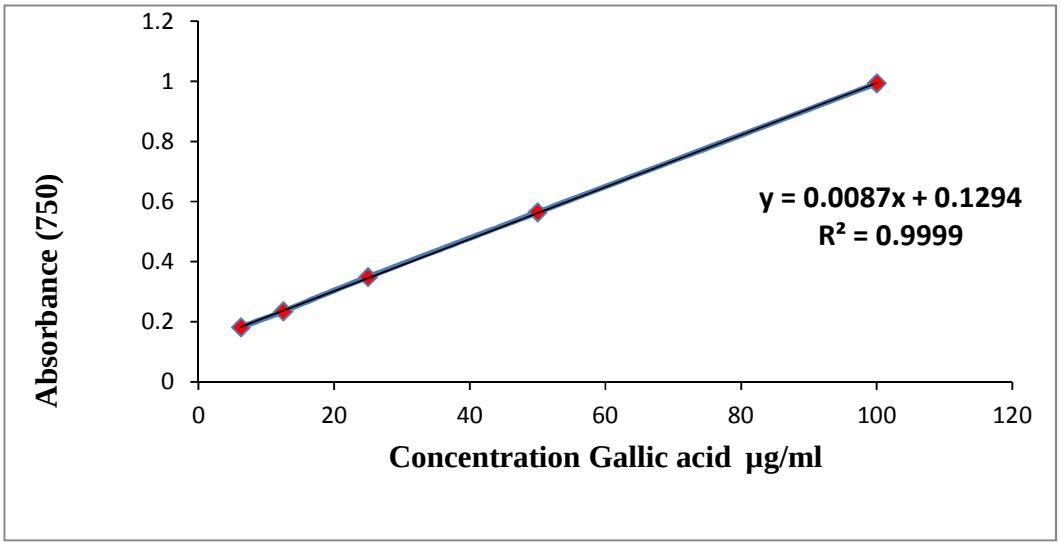


Fig 1: Standard Graph for Phenol

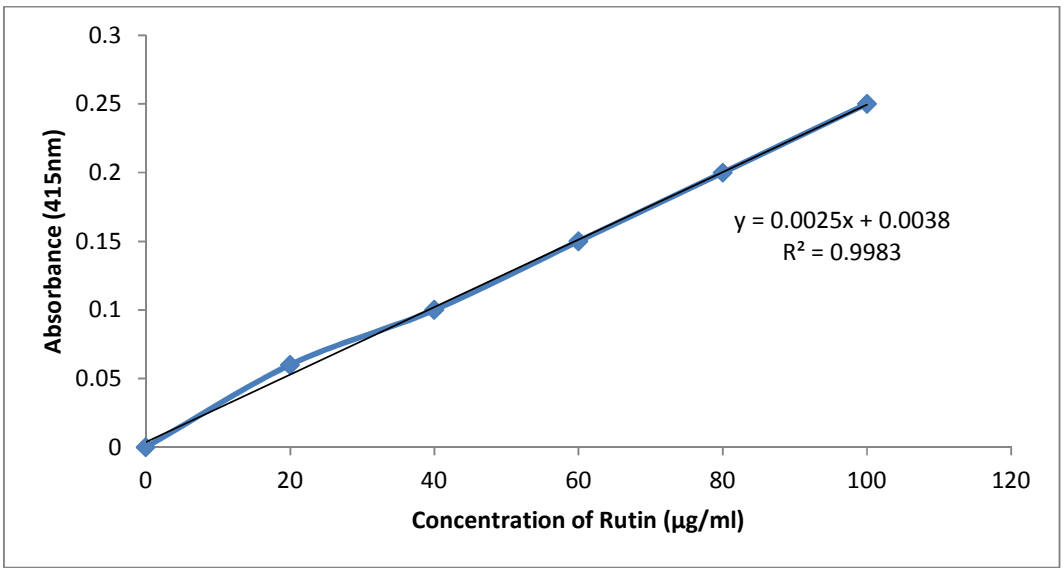


Fig 2: Standard Graph for Flavanoid

Table: 2 Total phenolic and Flavonoid

Sample	TPC (mg gallic acid/100 ml)	TFC (mg rutin/100 ml)
Ethanolic	34.66±1.288526	81.44±1.325041
Hydroalcoholic	78.48±0.900907	108.48±1.056141

***In vitro* Antioxidant Activity**

DPPH radical scavenging activity: The antioxidant activity of different concentration of *Bambusa arundinacae* was determined. The results showed that the extracts possess antioxidant activity. The free radical scavenging effect is increased with the increase in concentration. The IC_{50} value of hydro alcoholic extract is (405µg/ml) high when compared to ethanolic extract (266 µg/ml).The antioxidant value for ascorbic acid was determined to be 155.5µg/ ml.This shows that the antioxidant effect of hydro alcoholic extract is less than the concentration of ascorbic acid.

Total Antioxidant capacity: The assay is used to measure the amount of total antioxidants present in the plant extracts. The assay is a quantitative one since the activity is expressed as numbers of equivalents of ascorbic acid. Comparison of the total antioxidant capacity of the extract to standard ascorbic acid the antioxidant activity of the extracts exhibited increasing trend with the increasing concentration. While the standard antioxidant also showed the same trend as the concentration increased the activity also increased. The IC_{50} value of the hydro alcoholic extract is 0.043±0.002 and the ethanolic extract is 0.083±0.003µg/ml. This shows that the hydro alcoholic extract is comparably high when compared to the standard and the ethanolic extract.

Hydrogen peroxide scavenging activity: The OH radical scavenging assay shows the ability of ethanolic and hydro alcoholic extracts to inhibit OH radical mediated deoxy ribose degradation reaction mixture. The results are shown in the tab 3. The IC_{50} values of the ethanolic and hydro alcoholic extract were 50.39±1.477 µg/ml, 35.42±0.55µg/ml respectively. The IC_{50} value for ascorbic acid is 41.12±0.69 µg/ml. Hydro alcoholic extracts of *Bambusa arundinacae shoots* showed IC_{50} with higher concentration when compared to the standard.

Superoxide scavenging activity: In superoxide anion scavenging assay, ethanolic extract showed maximum superoxide anion scavenging activity and the results are presented in Table 3. The IC_{50} value of hydro alcoholic extract (67.33±0.763) was higher than ethanol (95.89±1.846).The two extracts suppressed deoxyribose degradation in a concentration-dependent manner. Ascorbic acid was used as reference standard. In this assay the IC_{50} value of Ascorbic acid was more than the two extracts. The hydro alcoholic extract showed higher activity when compared to the standard ascorbic acid.

Lipid peroxidation activity: The lipid peroxidation inhibition activity of *Bambusa arundinacae* was compared with standard ascorbic acid. The IC_{50} of Ethanolic and hydroalcoholic extract exhibited inhibition at 250µg/mL, respectively (Tab. 3). Both the extract showed almost moderate inhibitory activity to that of Ascorbic acid. A positive correlation of lipid peroxidation inhibition with free radical scavenging activities was observed.

Table 3: Antioxidant activity of *Bambusa arundinacae*

Name of the extracts	DPPH	Total antioxidant capacity(TAC)	Hydrogen Peroxide (H_2O_2)(IC_{50})	Superoxide dismutase (SOD)	Lipid peroxidation (LPO)
Ethanolic extract	405±1.03	0.083±0.003	**50.396±1.477**	95.89±1.846	49.123±1.022
Hydroalcoholic extract	266.7±4.37	0.043±0.002	35.42±0.5585	67.37±0.950	48.55±1.502
Aspirin	150.5±1.2	1.02±0.4220	41.12 ±0.691	100.5±0.693	50.23±0.9381

Each value is expressed as mean±SD n=3

Anti inflammatory activity

Protein denaturation method: In the present investigation, the in vitro anti-inflammatory effect of ethanol and hydro alcoholic extracts of bambusa arundinacae was evaluated against denaturation of egg albumin. The results are summarized in Table 4. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by ethanol and hydro alcoholic extracts of *Bambusa arundinacae* throughout the concentration range of 1000 to 15.625 µg/ml. Ibuprofen (at the concentration range of (1000 to 15.625µg/ml) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation. Ethanol and hydro alcoholic extracts possessed IC_{50} value 700 µg/mL and 212 µg/mL at the concentration range of 125 µg/ml whereas that of

Ibuprofen was found to be 118.33 µg/mL. However, the effect of Ibuprofen was found to be more when compared with ethanol and hydro alcoholic extracts of *Bambusa arundinacae.*

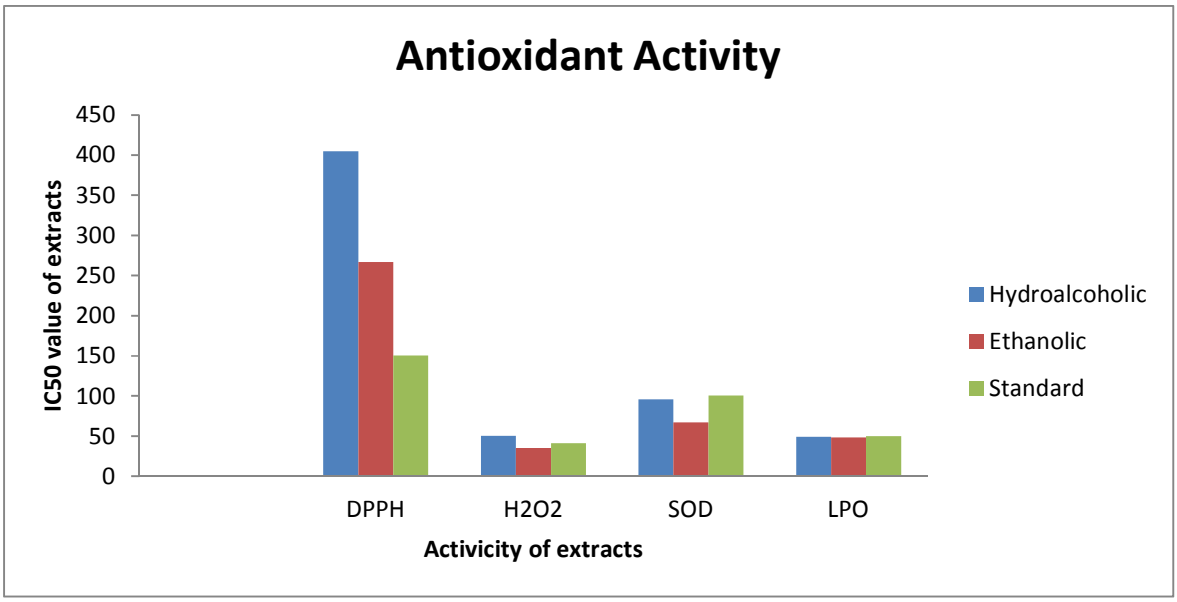
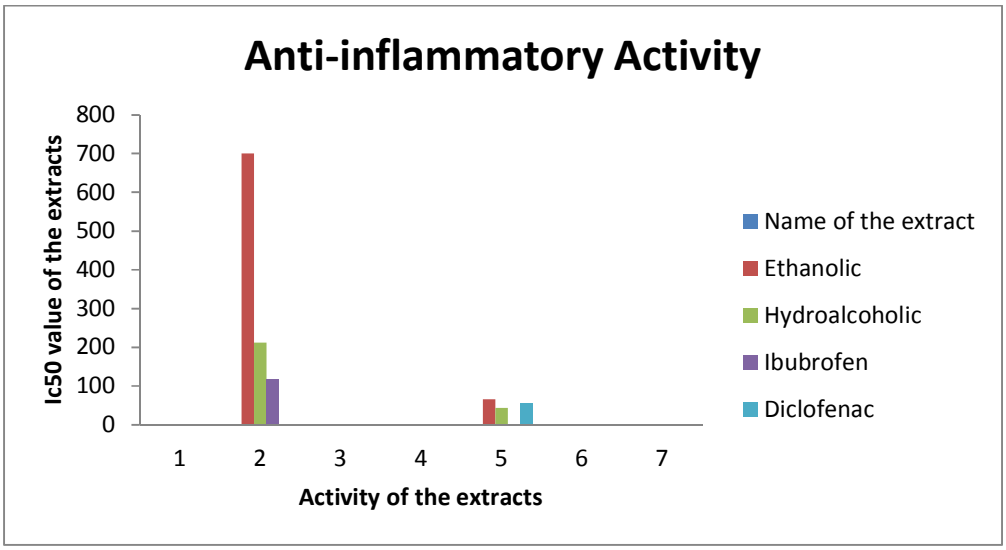


Table: 4 Antiinflammatory activity of Bambusa arundinacae (IC_{50} value)

Name of the extract	Protein denaturation	Membrane stabilization
Ehanolic extract	700±2	65.41±0.85687
Hydroalcoholic extract	212±2	44.29333±0.632561
Ibuprofen	118.3333±0.946	-
Diclofenac	-	55.562±0.5513



HRBC Membrane stabilization method: Ethanolic and hydro alcoholic extracts of *Bambusa arundinacae* prevent hypo tonicity induced membrane lysis (HRBC membrane stabilization method) to extent of 65.41 and 44.2 respectively at the concentration of 500 µg/ml which is comparable to that of the standard drug Diclofenac sodium 55.56 (500µg/ml). The anti-inflammatory activity of the both methanolic and hydroalcoholic extracts was concentration dependent

DISCUSSION

The phenolic and flavonoid compounds in plants contain hydroxyl group, which act as a good scavengers and are known as powerful chain breaking antioxidants[23]. They are a group of polyphenolic compounds which contain several biological properties such as anti-inflammatory, antihepatotoxic, antiulcer and anticancer.Plant phenols are vital nutritional antioxidants acquire an ideal structural chemistry for free radical scavenging activity.The present study indicated that the hydroalcoholic extract have good scavenging activity when compared to ethanolic extract.

DPPH radical scavenging activity is one of the widely used preliminary test for the plant extract[24].It is the direct and reliable method for determining radical scavenging action[25].Transfer of an electron or a hydrogen atom of antioxidants to DPPH neutralizes its free radical character[26]. In the present study the percentage of scavenging effect of DPPH radical was concomitantly increased with the increase in the concentration of both the extracts .From the results it is known that the species of *Bambusa arundinacae* possess hydrogen donating capabilities for hydroalcoholic extract and does free radical scavenging .Furthermore it was noticed that hydroalcoholi extract has more pronounced scavenging activity than the ethanolic extract of the standard ascorbic acid.

The total antioxidant activity of the extracts was measured spectrophotometrically based on the development of phosphomolybdenum complex[27]. The assay is effectively used to enumerate the total antioxidants present in the plant extracts. The method is a quantitative one since the activity is expressed as numbers of equivalents of ascorbic acid. The study demonstrated that the antioxidant activity of the extracts showed increasing trend with the increasing concentration of the plant extract. While the standard ascorbic acid also showed the same tendency as the concentration increased, the activity is also increased

Hydrogen peroxide being a weak oxidizing agent oxidizes the essential thiol (-SH) groups and directly inactivates a few enzymes. It can easily traverse cell membranes and rapidly enter inside the cell. H_2O_2 perhaps reacts with Cu^{2+} and possibly Fe^{2+} ions to form hydroxyl radical which is the source of many of its toxic effects[28]. It is therefore cells are biologically beneficial to control the accumulation of hydrogen peroxide. "*Bambusa arundinacae*" scavenged H_2O_2 and this may be credited to the existence of phenols and tannins which could donate electrons, thereby neutralizing it into water. It was observed that hydroalcoholic extracts of "*Bambusa arundinacae"* inhibition is high when compared to ethanolic extract.

Superoxide anions induce oxidative damage in lipids,proteins and DNA[29] by the formation of reactive oxygen species such as hydrogen peroxide hydroxyl radical and singlet oxygen[30,31].From molecular oxygen the superoxide are produced via nonenzymatic reaction by oxidative enzymes this study reveals that the hydro alcoholic extract of *Bambusa arundinacae* has the potential to scavenge superoxide anions when compared to the ethanolic extract.

Lipid per oxidation caused by reactive oxygen species induce membrane damage by peroxiding lipid moieties, particularly the polyunsaturated fatty acids[32].Second radical which is formed in the initial reaction reacts with the macromolecule, generating chain reaction and causing cellular abnormalities. Lipid per oxidation is considered one of the most important index of antioxidant activity. Here, lipid per oxidation inhibition activity of hydro alcoholic extract was higher than other extractives. These results demonstrate that *Bambusa arundinacae* can prevent cellular abnormalities caused by free radicals by breaking down the chain reactions responsible for lipid per oxidation.For treating several diseases caused by free radicals the *Bambusa arundinacae* is considered as a good source of natural antioxidants.

Protein denaturation bioassay method is an in-vitro method to evaluate the potential anti-inflammatory activity of the plant extracts. It is a recognized fact that denaturation of protein leads to inflammation and arthritic diseases [33].Preventing protein denaturation by plant products, would be a worth full research for anti-inflammatory drug therapy. The standard drug diclofenac sodium and the extracts exhibited dose dependent percentage inhibition of heat induced denaturation in fresh egg albumin. In protein denaturation method, percentage inhibition with respect to control is a measure of protein stabilization[34].Though the hydro alcoholic extract of bambusa arundinacae showed moderate free radical scavenging activity, its effect on inhibition of protein was found to be better than the ethanolic extract and was comparable with the standard diclofenac sodium.

The HRBC membrane stabilization method is a reliable *in vitro* method to know the anti inflammatory activity. The erythrocyte membrane is similar to that of the lysosomal membrane. Lysosomal stabilization is important in

preventing the release of lysosomal constituents, such as bacterial enzymes and proteases, which further causes tissue inflammation and damage upon extra cellular release. Various disorders are caused by inflammation due to the release of the lysosomal enzymes. The non steroidal drugs stabilize the membrane by inhibiting the lysosomal enzymes. Stabilization of RBCs membrane[35] further establishes the mechanism of anti-inflammatory action of different extracts of *Bambusa arundinacea.* Both the extracts were shown inhibiting the heat induced haemolysis compared with standard Aspirin. Membrane stabilization is an additional mechanism to provide the results for the anti inflammatory effect.

CONCLUSION

In conclusion, here I reported that the *in vitro* assays revealed that the plant extracts contain sufficient amount of phenols and flavonoids which act as an important source of natural antioxidant and anti inflammatory activity which might be useful as preventive agents against oxidative stress and inflammation. The present study provided evidence that the hydro alcoholic extract had potentential therapeutic activity compared to the ethanolic extract. Hence currently the *in vivo* evaluations of these extracts are in progress. Further studies should be carried out with the isolated compounds.

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