Evaluation of Antioxidant, Anti-inflammatory and Antimicrobial Properties of *Basella alba* Whole Plant

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Abstract: The present investigations were undertaken to validate the anti-inflammatory, antioxidant, and antimicrobial activity of the plant *Basella alba.* This methanolic extracts of *Basella alba* whole plant was analyzed for phytochemical constituents, antioxidant assays (DPPH, Reducing power and Hydrogen peroxide) and tested for inhibition against membrane stabilization protein and proteinase denaturation. The sample exhibited higher potential against the free radial especially the activity to scavenge the DPPH radical was found to be high as compared against reducing power and hydrogen peroxide assay. *In vitro* anti-inflammatory assays resulted in providing the strong inhibition against the protein and proteinase denaturation, when compared to the reference drugs Diclofenac in terms of activity. The extract exhibited excellent antimicrobial against bacteria and fungus using the agar well diffusion method which proves the plant besides provides activity against inflammation and oxidative stress can also effectively fight against the microorganism. Thus, *Basella alba* whole plant can act as agents for treating inflammatory conditions and infections hence it can be utilized more effectively in pharmaceutical industries.

***IndexTerms* -** *Basella alba*; Phytochemicals: Anti-inflammatory: Antioxidant: Antimicrobial activity.

I. Introduction

Man has been influenced with the challenge of disease and ailments; if these are not managed effectively it may lead to the extension of life threatening diseases. (Rang et al, 2007). About 80% of the world's population rely solely on traditional herbal practitioners for majority of their health needs (Amponsah et al, 2013). Therefore, there has been a recent surge of interest in traditional and alternative medicine both in the developed and in developing countries. Inflammation is an underlying cause or precipitating factor for most diseases in man including wounds, rheumatoid arthritis, some carcinomas and dermatological diseases (Erlinger et al, 2004). Though the current conventional therapy for inflammation (mainly non-steroidal antiinflammatory drugs (NSAIDs) and Steroids) is effective, the use of steroid has been influenced with numerous other drugs including gastritis, hypertension, diabetes and obesity (Kyei et al, 2012) which give adverse effects to the patients when used for the long term. Hence, there is a need for safer and effective therapy to replace this toxic effect of the drugs.

Many medicinal plants have been utilized worldwide as a herbal remedies for pain and inflammatory diseases (Boakye-Gyasi et al, 2008). Based on ethnopharmacological information the plant *Basella alba* was taken for the study (Cos et al, 2006). A number of pharmacological studies have indicated that by using *Basella alba* L.various diseases can be cured. It has shown hematological action, antioxidant activity, androgenic activity (Moundipa et al, 2008), the leaf extract of the *Basella alba* L is reported for the anti-inflammatory activity (Kumar et al, 2011), physiologically important sterols and sterol glucosides (Satya et al, 2010) have also been isolated from *Basella alba.*

Therefore based on the fact that although conventional medicines have been scientifically proven to be therapeutically potent, they are very expensive, sometimes inaccessible and have been credited with many of the adverse events associated with drug treatments (Houghton, 1995). Traditional herbal medicines have been in use as alternative remedies and have been ascribed with claims of efficacy and safety. A major drawback however of these alternative systems is the lack of existing scientific evidence to back and validate its claims of efficacy and safety (Owusu, 2009). Therefore research into plant medicines particularly based

on ethnopharmacological information is a logical venture in the search for new drugs (Patrick-Iwuanyanwu et al, 2011). Hence the study was undertaken to ascertain the medicinal value of the whole plant *Basella alba* to treat against various types of oxidative stress and inflammation.

II. Materials and Methods

2.1 Sample collection

Basella alba fruit were collected from Coimbatore, Tamilnadu, India during the month of January 2019. The whole plant was dried in hot air oven at 50ºC overnight and the samples were powdered and stored in zip lock covers.

2.2 Extraction

The dried parts of the plant was used for extraction using ethanol separately by microwave method (two-cycle method for 5 min) according to method of Adedapo et al. (2008). The extracts were filtered using Buckner Funnel and Whatmann No1 filter paper. The filtrate was further concentrated to dryness at 40°C using a rotary evaporator. Each extract was resuspended in the solvent ethanol to yield a 50mg/ml stock solution.

2.3 Phytochemical Screening (Azad et al, 2013)

2.3.1 Tests for Alkaloids

2.3.1.1 Mayer's Test: 2ml solution of the extract and 0.2 ml of dilute di-hydrochloric acid were taken in a test tube. Then 1ml of Mayer's reagent was dissolved. Yellow color precipitate was formed and that was indicated as the presence of alkaloids

2.3.1.2 Dragendroff's Test: 2ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a test tube. Then 1ml of Dragendroff; s reagent was added. The appearance of orange brown precipitate indicated the presence of alkaloids

2.3.2 Tests for Tannins

2.3.2.1 Ferric Chloride Test: To the extract (5ml), 1ml of 5% ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins

2.3.3 Test for Flavonoids: Added a few drops of concentrated hydrochloride acid to a small amount of an alcoholic extract of the plant material. The presence of flavonoids was confirmed by the immediate development of red color.

2.3.4 Tests for Saponins: 1ml solution of the extract was dilute with water to 20ml and shaken in a graduated cylinder for 15minutes. No one-centimeter layer of foam indicates the absence of saponins.

2.4 Total Polyphenols Content

Total phenolic content of the organic solvent extracts of different plants was determined with Folin-Ciocalteu reagent method (Mc Donald et al, 2001). 0.5mL sample (1mg/mL in distilled water) was taken in triplicates and 0.1mL Folin-Ciocalteu reagent (0.5 N) was mixed and then the incubated for 15 minutes at room temperature. Then 2.5mL of saturated sodium carbonate was added and mixture was again incubated for 30minutes at room temperature. The absorbance of the mixture was measured at 760nm. Gallic acid was used as a standard. Total phenol values were expressed in terms of Gallic acid equivalent (mg g-1 of extracted compound).

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2.5 Total Flavonoid Content

Flavonoids were estimated by a modified method of Lallianrawna et al, (2013). To 1 ml of sample, 75µl of 5% $NaNO_2$ solution was added. After 5 min, 150µl of 10% $AlCl_3.6H_2O$ was added to the mixture, which was kept at room temperature for 5 more minutes. This was followed by the addition of 0.5 ml of 1M NaOH and the total volume was made up to 2.5 ml with the addition of deionised water. The resulting solution was mixed well and immediately, the absorbance was measured at 510nm on a UV-VIS spectrophotometer. For the blank, the extracts were replaced with an equal volume of deionised water. Total flavonoid content of the samples was expressed as the mg equivalent to catchin /g of extract.

2.6 Antioxidant assays

2.6.1 Scavenging activity of DPPH radical

Scavenging activity of betacyanins against DPPH radicals was assessed according to the method of Liyana-Pathirana and Shahidi (2005) with some modifications. Briefly, 0.1mM DPPH-methanol solution was mixed with 1ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30min at 25ºC in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

DPPH-scavenging activity (%) = [1-(absorbance of the sample – absorbance of blank)/absorbance of the control] *100

2.6.2 Hydrogen Peroxide Scavenging Activity

A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4).0.2ml of various concentration of *Basella alba* betacycanins (1, 10, 50, 100mg/ml) in 1.6ml phosphate buffer (pH 7.4) was added to 0.6ml of 40mM hydrogen peroxide solution. The absorbance value of the reaction mixture was recorded at 230 nm (Lcin, 2005). The percentage of hydrogen peroxide scavenging of *Basella alba* betacyanin was calculated using the following formula:

Hydrogen peroxide scavenging activity % = [(absorbance of the sample- absorbance of the control)/ absorbance of the control]*100

2.6.3 Determination of reducing power

The reducing power assay was determined by the modified method of Oyaizu (1986). A 0.25ml aliquot of *Basella alba* betacyanin evaporated samples in the concentration of 1mg/ml, 10mgml, 50mg/ml, and 100mg/ml was mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50ºC for 20min. After 2.5ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650g for 10min. A 5ml aliquot of the upper layer was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride at 700nm was measured.

2.7 Anti-inflamatory assay

2.7.1 Human RBC's membrane stabilization method

The human red blood cells (HRBC) membrane stabilization method was adopted from Shinde et al, 1999. Blood was withdrawn from healthy volunteers and it was mixed in equal volume with Alsever's solution (2% dextrose, 0.8% sodium nitrate, 0.05% citric acid and 0.42% NaCl). Ten v/v suspension of RBC was made by using isosaline (0.85% NaCl). Following, samples at 1mg/ml concentration were mixed with 1ml of phosphate buffer (0.15M, pH7.4) and 2ml of hyposaline (0.36% NaCl). Subsequently, 0.5ml of HRBC suspension was added and incubated at 58°C for 30min. Blank was taken as phosphate buffer. The standard taken was diclofenac. This experiment was conducted in triplicates. The absorbance was measured at wavelength 560nm by UV-Vis spectrophotometer. The control was RBC in isosaline. The % protection of HRBC membrane stabilization was estimated by the following formula:

$$\text{\% protection of HRBC membrane stabilization} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

2.7.2 Inhibition of protein denaturation assay

A reaction mixture consists of various concentrations of plant extract 1000 µL (100- 500 µg/ml), 200 µL of egg albumin or 450 µL (5% w/v aqueous solution) bovine serum albumin, 1400 µL of phosphate buffered saline. Distilled water instead of extracts with above mixture is used as a negative control. Afterward, the mixtures is incubated at 37 °C for 15 min and then heated at 70°C for 5 min. After cooling under running tap water, their absorbance is measured at 660 nm. diclofenac sodium is taken as a positive control (Banerjee et al, 2014). The experiment is carried out in triplicates and percent inhibition for protein denaturation is calculated using following equations:

% Inhibition of denaturation = (1-D/C) X100

Where D is the absorbance of test sample and C is the absorbance of negative control (without the test sample or reference drug).

2.7.3 Assay of proteinase inhibition

In this assay different enzymes and different protein can be used, enzymes are proteinase are used as protein. The reaction mixture (2 ml) contain 0.06 mg proteinase (1 ml 20 mM TrisHCl buffer (pH 7.4) and 1 ml test sample/ standard drug, Diclofenac sodium, of different concentration 100-600 g/ml. The mixture is incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein or 4% (w/v) bovine serum albumin is added. The mixture is incubated for an additional 20 min. 2 ml of 5% trichloroacetic acid (TCA) is added to terminate the reaction. Cloudy suspension is centrifuged at 3000 rpm for 10 minutes or 2500 rpm for 5 minutes and the absorbance of the supernatant is read at 210 nm or 217 nm against buffer as blank (Sakat et al, 2010). The experiment is performed in triplicate. The percentage inhibition of proteinase inhibitory activity is calculated using the following equation.
Percentage inhibition = (Abs control –Abs sample) X100/ Abs control.

2.8 Antimicrobial assay

2.8.1 Micro-organisms: *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus, Aspergillus niger and Aspergillus fumigatus* were the microorganisms used and they were stored at freeze temperature until use. To prepare 24 hours Pure Culture: A loop of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 hours for bacterial culture and 48 hours in case of fungal culture.

2.8.2 Agar-well Diffusion Method:

Using 25ml of sterile Nutrient agar medium (bacterial culture) or Sabouraud agar medium (fungal culture) was poured into sterile culture plates and allowed to set. 0.5ml of 24 hours old culture of test organism was layered onto the medium and allowed to set. The seed medium was then allowed to dry at room temperature for about 30 minutes 13. With the aid of a sterile cork borer, wells of about 8mm in diameter were punched on the plates. About 0.5ml of each dilution of the extracts, 0.5ml of streptomycin and nystin (positive control) and methanol (negative control) was dispensed into the wells and the plates were incubated at 37ºC for 24 hours for bacterial cultures and for fungal culture it was incubated at room temperature for 48 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm (Orisakeye and Olugbade, 2012).

III. RESULT AND DISCUSSION

3.1 Phytochemical Analysis:

The phytochemical screening of *Basella alba* extracts showd the presence of tannin, flavonoids and alkaloids and absence of saponins (Table 1). The phytochemicals present in the natural substances is responsible for the medicinal and biological activities of the plant. Steroids and triterpenoids displayed analgesic and anti- inflammatory properties (Sakat et al, 2010). The presence of biologically important phytochemicals in Chetoui O. europaea variety has contributed to their medicinal value, hence presence of phytochemical act as a potential source for useful drugs.

Table 1. Phytochemical Analysis of *Basella alba*

Phytochemical	Result
Alkaloids	+
Flavonoids	+
Tannin	+
Saponins	-

3.2 Total Phenolics and Flavonoids contents.

Total phenolic content (TPC) was determined using the Folins reagent and expressed in terms of mg / 100 ml extract. The TPC content of the extract was found to be 1.12g/100g equivalent to gallic acid. In case of flavonoid the content was 460mg/100g (Fig. 1). The extract possessed higher content of the bioactives (phenolics and flavonoid are the which are known for its potential source of natural antioxidants and also possess other biological properties such as inhibition of hydrolytic and oxidative enzymes, anti-inflammatory and against cancer and cardiovascular diseases (Münzel et al, 2010). In addition to their individual effects, bioactives in interact synergistically to provide several other beneficial health effects.

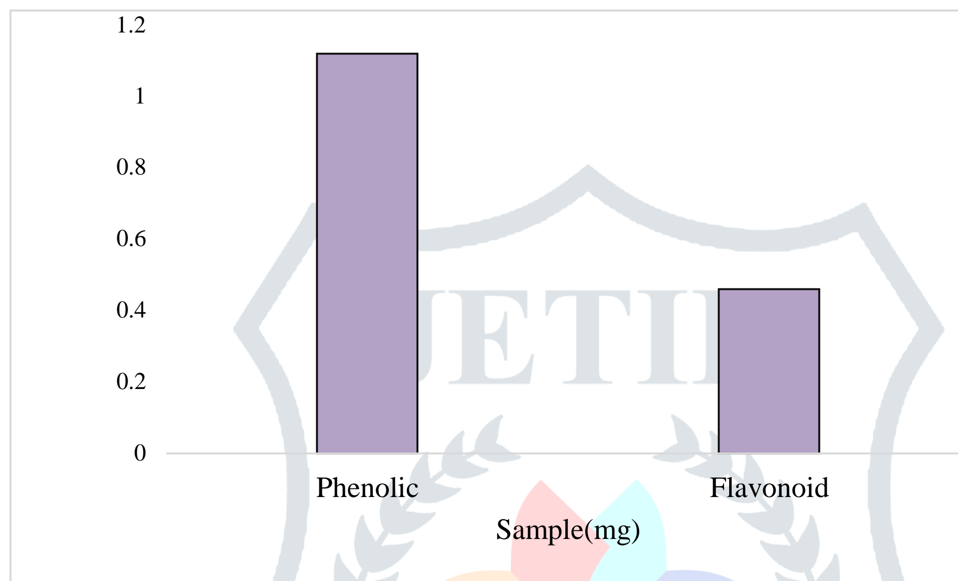

Figure 1. Total Phenolic and Flavanoid content of *Basella alba*

3.3 Antioxidant Analysis

3.3.1 DPPH radical scavenging activity

DPPH is a compound and has been used widely to test the free radical scavenging ability of various samples (Fig. 2). The ability of phenolic compound to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging assay. Antioxidant activity of sample extracted in acidified methanol. At 1mg/ml the scavenging effect was found to be 36%. The scavenging activity of the sample increased with the increasing concentration of the sample. It shows that *Basella alba* fruit betacyanin have strong hydrogen donating capacity and can be effectively scavenge DPPH radicals. These results indicate that radical scavenging capacity of betacyanin might be mostly related to there concentration of phenolic and hydroxyl group. The antiradical activity of phenolic compound depends on their molecular structure on the availability of phenolic hydrogen and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation (Catherine et al, 1996).

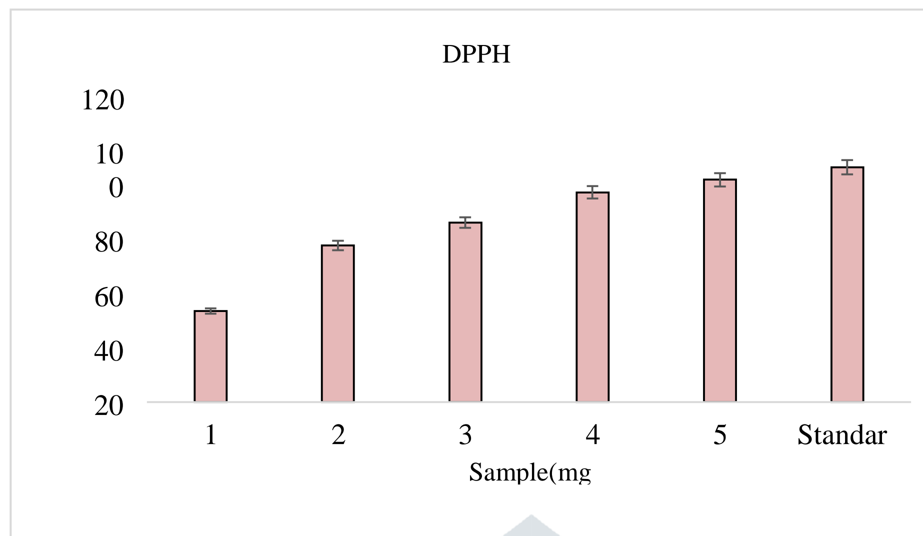

Figure 2. DPPH activity of *Basella alba*

3.3.2 Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activities of the extracts, BHT was measured at 450nm. Hydrogen peroxide scavenging activities of the extracted was 36%at 1 mg/ml, 52% at 2 mg/ml, 70% at 3 mg/ml, 79% at 4mg/ml and 85% at 5 mg/ml respectively. BHT (94%) had higher hydrogen peroxide scavenging activity that the extracts (Fig. 3). It was reported earlier that the extract were capable of scavenging hydrogen peroxide in concentration depend manner. Although hydrogen peroxide itself is not reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus removing H2O2 is very important for food system. It extract were capable of scavenging hydrogen peroxide in concentration depend manner. Although hydrogen peroxide itself is not reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell (Nabavi et al, 2008). Thus scavenging H2O2 is very important for food system.

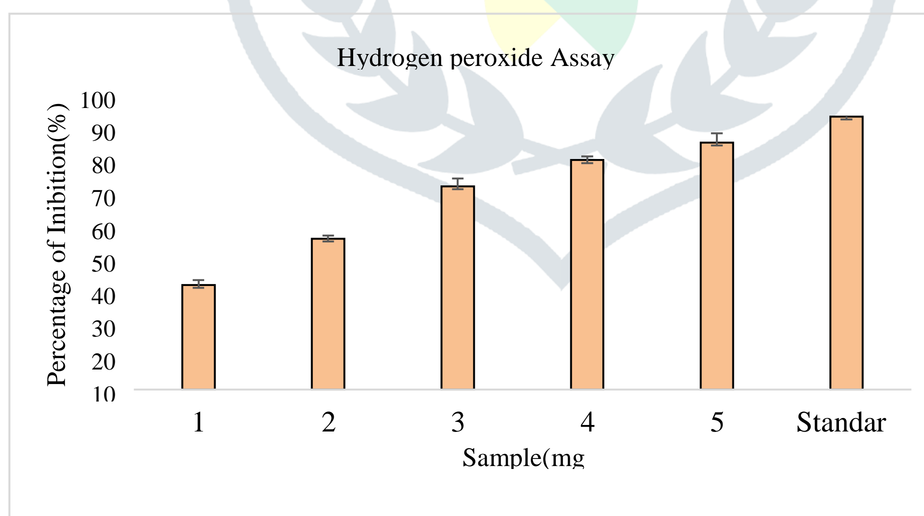

Figure 3. Hydrogen Peroxide assay of *Basella alba*

3.3.3 Reducing power

It has been reported that reducing power is associated with antioxidant activity and may serve as significant reflection on the antioxidant activity of the whole plant *Basella alba*. The plant exhibited a higher reducing power than butylated hydroxyl toluene, suggesting that strong electron-donating capacity. The

reducing power of *Basella alba* at 1 mg, 2mg, 3mg, 4mg and 5mg was 0.22, 0.44, 0.77, 0.84,1 respectively (Fig. 4). The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by denoting a hydrogen atom. The transformation from Fe3+ to Fe2+ occurs in the presence of the extract because iron has the ability to gain and loss electrons very easily, thus it acts as a common catalyst of oxidation reaction. The reducing power activity of *Basella alba* fruit is may be probably due to the presence of phenolic compounds which might act as electron donor.

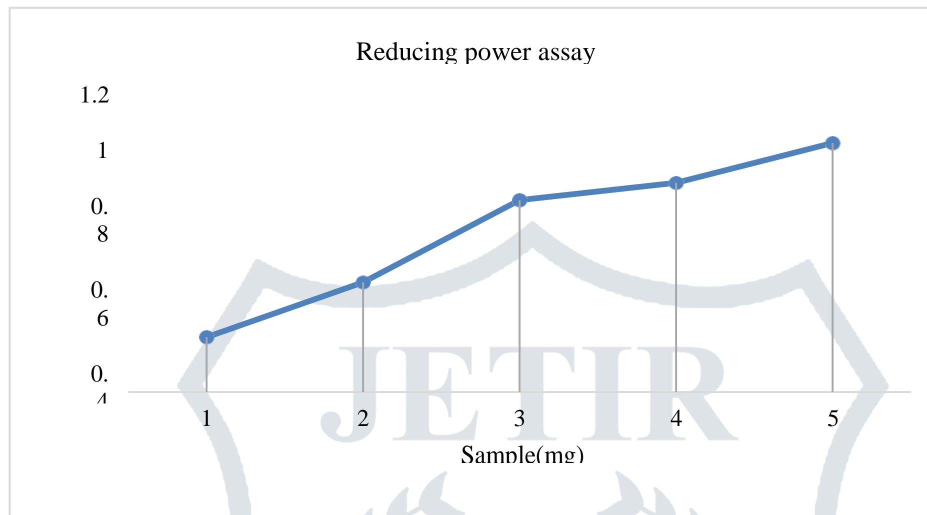

Figure 4. Reducing power assay of *Basella alba*

3.4 Anti inflammatory Assay

3.4.1 Membrane stabilization assay

The anti-inflammatory activity of the extracts was concentration dependent manner, with the increase in concentration, the activity is also increased ranging from 30.67% to 96% for 1 to 5mg respectively (Fig. 5). Human red blood cell membranes are similar to lysosomal membrane, the inhibition of hypotonicity and heat induced lysis of red blood cell membrane will be taken as a measure of the mechanism of anti-inflammatory activity. During inflammation, lysis of lysosomal membrane may occur which release their enzyme components that produce a variety of disorders. Non-steroidal anti-inflammatory drugs (NSAIDs) produce their effects by either inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes.

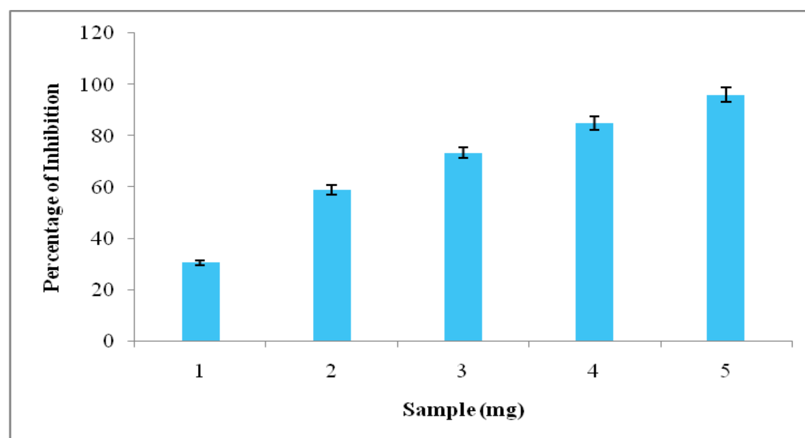

Figure 5. Membrane stabilization assay of *Basella alba*

3.4.2 Protein Denaturation

Denaturation of proteins was well documented cause of inflammation. As part of the investigation on the mechanism of the ant inflammation activity, ability of extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation Maximum inhibition of 85% was observed at 5mg/ml concentration with the IC50 value of 1.254mg/ml (Fig. 6). Anti-inflammatory assay was performed by denaturating the protein by keeping the reaction mixture at 70°C in a water bath for 10 minutes. Protein denaturation results loose of biological properties of protein molecules. Protein denaturation has been correlated with the formation of inflammatory disorders like rheumatoid arthritis, diabetes and cancer. The neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage The plant extracts acts as a anti-inflammatory compound by inhibiting the release of lysosomal content from neutrophils at the site of inflammation. Hence ability of substance to prevent the protein denaturation may also help to prevent the inflammatory disorders (Sangeetha & Vidhya, 2016).

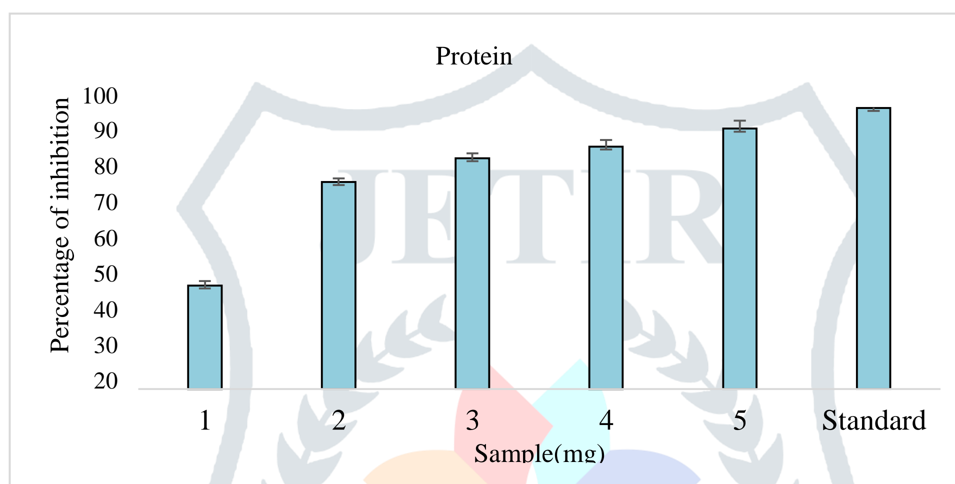

Figure 6. Protein denaturation assay of *Basella alba*

3.4.3 Proteinase inhibitory activity

The *Basella alba* extract exhibited significant anti proteinase activity. The percentage of inhibition ranged from 35% to 88% at 1 to 5mg/ml concentration. The standard diclofenac (positive control) showed inhibition percentage of 95% at 1mg/ml (Fig. 7). Proteinases have been implicated in arthritic reactions. During inflammatory reactions it was reported previously that leukocytes proteinase plays a major role in the development of tissue damage and significant level of protection was provided by proteinase inhibitors (Das & Chatterjee, 1995). Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the antioxidant and antinflammatory activities of many plants that correlates well with our results.

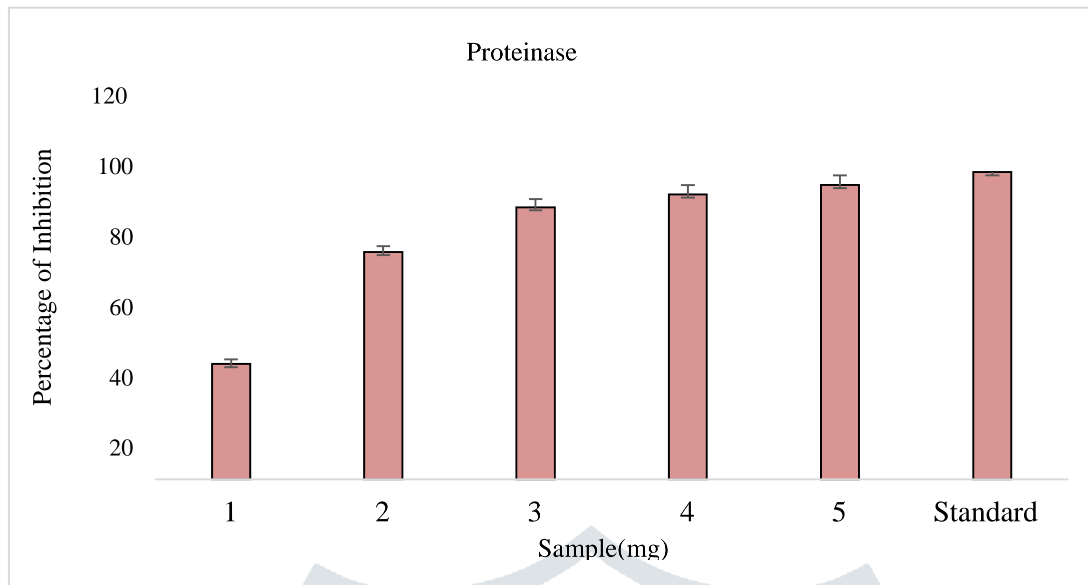

Figure 7. Proteinase inhibition of *Basella alba*

3.5 Antimicrobial assay

The antimicrobial activities of ethanolic extract of the whole plant of *Basella alba* gave different zones of inhibition on the organisms tested .The extract inhibited the growth of all most all the bacteria isolates. The highest zone of inhibition was observed in *Bacillus* with 1.30mm at 100mg concentration followed by *Candida* with 1.20mmat 100mg concentration. *Pseudomonas, Klebsiella pneumoniae, E.coli* showed moderate zone of inhibition with 0.70mm, 0.90mm,0.70mm at 100mg concentration.(Table 2). The results was observed in the extract from *Basella alba* indicating that gram positive strain was more sensitive then gram negative. This observation can be attributed in the difference in the structure of bacterial cell wall. The less complex structure of the cell wall in the gram positive bacteria makes it more permeable to the antimicrobial compounds

Phenolic compounds such as chlorogenic, caffeic, p- caumaric, ferulic p-hydroxy benzoic, vanillic, protocatechuic, syringic well as some other phenolic compound like Quercetin, hydroxyl tyrosol, resveratrol identified to have antimicrobial activities (Reshmi et al, 2012). Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes, altering their function and structure, causing swelling and increasing their permeability. The increases in cytoplasmic membrane permeability appear to be a consequence of the loss of the cellular pH gradient, decreased ATP levels, and the loss of the proton motive force, which lead to cell death.

Table 2. Antimicrobial activity of *Basella alba*

Extract	Micro Organism	Concentration (mg/ml)	Zone of inhibition(mm)	Streptomycin (1mg/ml)
Basella alba	*Pseudomonas*	50 100	0.65±0.85 0.60±0.90	0.90±1.10
	Candida	50 100	0.40±0.60 1.10±1.30	1.30±1.50
	Klebsiella pneumoniae	50 100	0.50±0.80 0.60±0.90	1.10±1.30
	Bacillus	50 100	0.40±0.60 1.20±1.40	1.00±1.20
	E.coli	50 100	0.40±0.60 0.60±0.80	0.90±1.10

IV. Conclusion

From the above obtained results, it can be concluded that the whole plant *Basella alba* shows a significant anti-inflammatory activity which was demonstrated in above methods in a dose dependent manner. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development *Basella alba* plant against oxidative stress and inflammation. To utilize the whole plant of *Basella alba* in polyherbal formulation further studies are required in identifying the potential bioactive compound and analyzing its anti-inflammatory activity in animal models. Hence the medicinal plant by *in vitro* results appear as interesting and promising and may be effective as potential sources of novel antimicrobial, antioxidant and anti-inflammatory drugs.

Acknowledgments

We are grateful to Principal, Sri Ramakrishna College of Arts and Science for constant encouragement throughout the course of study

Conflict of interest

The author declares no conflict of interest

Reference

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