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Phytochemical, antioxidant and in-vitro anti-inflammatory studies of peels of sweet potato (Ipomea batatas) have shown a promising natural cure against antibiotic resistant bacteria and opportunistic fungus

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Abstract

Background- Multidrug-resistance has become an emerging health concern worldwide mostly in the immune-compromised patients. This indicates the need to search for alternative methods of controlling antibiotic-resistant pathogens. Ethono-medicinal data indicated sweet potato (*Ipomoea batatas*), a widely consumed vegetable as a potent remedy in inflammatory and infectious diseases. The present study evaluated the phytochemical, antioxidant and anti-bacterial action of the ethanolic extract of *Ipomoea batatas* peels against some drug-resistant pathogens like *Pseudomonas aeruginosa, Staphylococcus aureus* and anti-fungal action against *Aspergilus niger* responsible for most of the infections leading to the treatment failure. There is inflammation induced disorders like cardiovascular diseases, atherosclerosis, type 2 diabetes, etc. where the peels of *Ipomoea batatas* have very much potent anti-inflammatory activity.

Methods- The peels of sweet potato were authenticated and macerated ethanolic extract was used for the Phytochemical analysis, antioxidant, in-vitro anti-inflammatory studies were performed. The antifungal activity was studied against *Aspergilus niger* and antimicrobial action was evaluated against two most commonly found resistant strains like *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Results- The phytochemical analysis the ethanolic extract of the peels of *Ipomoea batatas* has shown the presence of alkaloid, glycoside, phenol and tannin. Antioxidant activity in terms of total phenolic & total flavonoid contents has shown a significantly good value, less than 50µg/ml. The anti-bacterial activity against two antibiotic resistant bacteria *Pseudomonas aeruginosa and Staphylococcus aureous* were prominently good with the MIC (Minimum Inhibitory Concentration) 25 mg/ml and for the fungal strain *Aspergilus niger*, the MIC was 50 mg/ml. The various in-vitro anti-inflammatory studies like heat induced haemolysis, protein denaturation study etc. have proved that the sweet

potato leaves have potent anti-inflammatory activity with an IC_{50} value less than 400 µg/ml.
Conclusion- The present study indicated the ethanolic extract of *Ipomoea batatas* peel can be beneficial to develop a cost-effective natural source of antimicrobial agent against drug resistant pathogen and also source of cure for various inflammation induced disorders. Further study will lead to a sustainable source in pharmaceutical industries also helpful to maintain an eco-friendly environment.

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Keywords: Sweet potato, Ipomea batatas, anti-inflammatory, Pseudomonas aeruginosa and Staphylococcus aureus.

Introduction:

In an era marked by the constant evolution of medical science, one timeless and enduring branch of healing continues to captivate attention and spark curiosity is herbal drugs. For centuries, the utilization of plants and their extract from parts including leaves, roots, flowers, and seeds, have recently attracted fresh interest because of their potential as alternative or supplementary therapies. As we explore deeper into the realm of herbal medicines, we find a vast library of organic substances with a variety of pharmacological effects. The potential of herbal medications presents a captivating story that links traditional knowledge with contemporary scientific study, ranging from antioxidant-rich mixtures that promote wellbeing to plants that hold complex bioactive compounds capable of targeting specific ailments.
The sweet potato (*Ipomoea batatas*) [Fig:1] is a dicotyledonous plant that belongs to the bindweed or morning glory family, convolvulaceae[1] Literature study says that commonly the sweet potato is a valuable medicinal plant having anti- cancer, anti-diabetic, and anti-inflammatory activities, antioxidant etc properties. Sweet potato is now considered a valuable source of unique natural products, including some that can be used in the development of medicines against various diseases and in making industrial products[2] The sweet potato is reported to be superior sources of polyphenols, terpenoids,glycosides, alkaloids, steroids, and other functional bioactive components the predominant bioactive components are phenolic compounds like phenolic acids (e.g., caffeic acid, monocafeoyl quinic (chlorogenic acid), caffeoyl quinic acid (CQA) derivatives, dicaffeoylquinic, ferulic, *p*-hydroxybenzoic, coumaric, sinapic, syringic, and vanillic acids)[3], flavonoids (Quercetin, myricetin, luteolin, kaempferol, and apigenin), anthocyanins (3-sophoroside-5-glucoside derivatives of peonidin, cyanidin, and pelargonidin aglycones, almost all of them mono- or di-acylated with *p*-hydroxybenzoic acid, ferulic acid, or caffeic acid)[4].

(a (b)

FIG 1: (A) *IPOMEA BATATAS* (SWEET POTATO) FRUIT (B) *IPOMEA BATATAS* (SWEET POTATO) LEAVES

Sweet potato is an herbaceous perennial vine that has white and purple sympetalous flowers, large nutritious storage roots, and alternate heart-shaped or palmately lobed leaves. The large, starchy, sweet-tasting tuberous roots are by far the most important part of the plant. Sweet potato roots are long and tapering, with smooth skin that ranges in color from yellow to orange to red to brown to purple to beige. Its flesh is beige, white, red, pink, violet, yellow, orange, and purple in addition to other colors[5].
The increasing concern over antibiotic resistance and the desire for sustainable solutions have led researchers to investigate a wide range of botanical sources for their inherent antimicrobial properties. One such source that has gained attention is the sweet potato (*Ipomoea batatas*), a widely cultivated root crop with a rich

nutritional profile. Beyond its role as a staple food, emerging evidence suggests that sweet potatoes may herbal compounds capable of combating various microorganisms, opening avenues for innovative antimicrobial strategies

At present world various inflammatory diseases including rheumatoid arthritis, Alzheimer's disease, asthma, cancer, Type-2 diabetes etc. are well spread throughout the World[6]. Having a natural source which can have anti-inflammatory properties can be very much useful. Flavonoids from sweet potato can work as anti-inflammatory compounds by passing through several pathways such as inhibition of cyclooxygenase (COX) and lipoxygenase enzyme activities, inhibition of neutrophil degranulation, inhibition of histamine increase, and inhibition of leukocyte accumulation[7].

As it is very much evident from the above discussion that the sweet potato has got promising anti-inflammatory, antioxidant, antimicrobial property thus the peels of sweet potato which is not usually consumed, generally goes on waste can be a good target to predict the above properties.

The primary aim of this study was to elucidate the antimicrobial potential of sweet potato peels to combat antibiotic resistance against antibiotic resistant strains, antifungal property against opportunistic infections and also in-vitro anti-inflammatory property against various inflammation induced diseases.

MATERIALS & METHODS

Collection and Authentication of Plant

The peels of *Ipomoea batatas* (about 3kg) were collected from local area plant during the month of September 2022 and the plant was identified by the help of regional nursery and finally authenticated by Botanical Survey of India, Howrah, West Bengal. The plant is identified with specimen no. JISU/PHARM.TECH/SSAHA-001 and also identified that *Ipomoea batatas* plant belong to family Convolvulaceae.

Extraction procedure

About 50 g of coarse powder was taken into a conical flask and 500 ml of ethanol (solvent) was added to the conical flask. Then it was wrapped in aluminium foil and allowed to macerate for about 5 days with periodical shakings. After cold maceration, the extract was filtered and the excess solvent was reduced using a rotary evaporator. The semisolid extract was refrigerated at 4°C for further analysis[8].

The extract was diluted using ethanol and made the concentration of 1mg/ml. This Solution has been utilized for various phytochemical analyses of secondary metabolites[9].

Phytochemical Screening

Phytoconstituents indicating the secondary metabolites of a plant helps in growth, defense mechanism and responsible for its morphological characteristics. The phytochemical screening of the extracts was performed to identify the main groups of chemical constituents (carbohydrates, alkaloids, glycosides, tannins, saponins, phenols etc) present in the extracts using the standard protocol[9].

Test for Carbohydrate

Molisch test- 2ml of extract treated with 2 ml of concentrated H_2SO_4 and few drops of Molisch reagent was added. Reddish brown ring will formed at junction of two layers, which confirmed the presence of carbohydrate.

Test for Alkaloid

1) Mayer's test- plant sample of 200 mg was taken in 10 ml ethanol and was filtered. In 2 ml filtrate 1% HCL was added and was steamed and 1 ml of filtrate was treated with 6 drops of Mayer's reagents. Formation of yellow colour precipitate indicates presence of alkaloid.
2) Dragondroff's test- Take 2 ml of filtrate treated with 0.1N HCl and adds few drops of Dragondroff reagent. Orange brown precipitate will formed. This also confirmed the presence of alkaloid in the plant sample.

Test for Reducing Sugar

Fehling's test: Take 5ml of ripe and unripe extracts separately and add 5ml of Fehling's solution (Fehling's A and B) by boiling for 2-5 minutes. Formation of brick red precipitation determines the presence of reducing sugar.

Test for Phenol

Few drops of 1% $FeCL_3$ were added to 3 ml of filtrate. Then 10% lead acetate solution was added. Yellow colour precipitate will formed if phenolic compounds are present.

Test for Glycoside

Keller Killani Test

1ml of extract treated with 4 ml glacial acetic acid and 1ml concentrated H_2SO_4, and add drop wise 2% FeCl3 to mixture. Brown ring formed at junction of liquid layer of mixture will confirm the presence of glycoside

Test for Saponin

Foam test: 2ml of extract along with 5ml of distilled water were mixed well. If foam persists for 10 minutes, saponin is present.

Test for Tanin

3 ml of extract was treated with 5% $FeCL_3$ solution. Greenish black colour precipitate confirms the presence of tannin.

Anti-Oxidant Activity

Total Phenolic content

The total phenol content was determined with the Folin-Ciocalteu's assay using gallic acid as standard. In the procedure, 0.5 ml of plant extracts were mixed with 1.5 ml Folin- Ciocalteu' s reagent (FCR) diluted 1:10 v/v than after 5 minutes 1.5 ml of 7% sodium carbonate solution was added. The final volume of the tubes was made up to 10 ml with distilled water and allowed to stand for 90 minutes at room temperature. Absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer[10].

Total Flavonoid content

Total flavonoid content was determined by aluminium chloride method using quercetin as a standard. 1ml of test sample and 4 ml of water was added to a volumetric flask (10 ml volume). Add 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added after 5 minutes. After 6 minutes of incubation at room temperature, 1ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. Absorbance of the sample was measured against the blank at 510 nm using a spectrophotometer[10].

Anti-Microbial Study

Determination of Minimum Inhibitory Concentration

At first all the apparatus required for the antimicrobial study should be autoclavedproperly. Distilled water used in the study is also sterilized. Nutrient broth is prepared. Five Concentrations (15, 25, 50, 75, 100 mg/ml) of the sample extract is prepared using 1% DMSO (Dimethyl sulfoxide). Then each of them is dissolved in 3ml of broth in test tube. One loop full of each bacterium and the fungus *Aspergilla's niger* were given to eachofthe test tubes. Then the test tubes are incubated for 24 hours at 37° C in BOD incubator. After 1 day of incubation, turbidity is checked for each test tube. The dilutions in which the solutions appear to be clear are regarded as the inhibitory concentrations. The Minimum concentration of sample at which the antimicrobial activity is observed i.e. the solution appears to be clear is taken as Minimum Inhibitory Concentration (MIC) of the sample for showing Anti-microbial activity. All the experimental procedure should be conducted inside an aseptic area[11].

Determination of Zone of Inhibition

Various Nutrient media (Mannitol Salt Agar for *S. Aureus*, Cetrimide Agar for *P. Aeruginosa* and Potato dextrose agar for *A. Niger*) were prepared. Zone of inhibition is determined by standard procedure[12].

Anti-Inflammatory study

Preparation of Phosphate Buffer (pH 7.4) along with goat blood samples for membrane stabilization assays is done.

Hypo tonicity-induced haemolysis

Sample extracts of various concentrations were prepared (100, 200, and 500 µg/ml), respectively using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of goat blood

suspension were added. It was incubated at 37 °C for 30 min and centrifuged 3000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. A control was prepared by omitting the extracts .The percentage inhibition of hemolysis or membrane stabilization was calculated[12, 13].

% inhibition of haemolysis = [(A1 – A2)/ A1] x 100 Where A1 = absorption of the control, and A2 = absorption of test sample mixture

Heat-Induced Hemolysis

0.05 mL of goat blood cell suspension and 0.05 mL of hydro ethanolic extracts of the leaves were mixed with 2.95 mL phosphate buffer (pH 7.4). The mixture was incubated at 54 °Cfor 20 min in a shaking water bath. After the incubation, the mixture was centrifuged (2500 rpm for 3 min), and the absorbance of the supernatant was measured at 540 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). Phosphate buffer solution was used as a control for the experiment[12].

% inhibition of hemolysis = [(A1 – A2)/ A1] x 100

Where A1 = absorption of the control, and A2 = absorption of test sample mixture

Effect on Protein Denaturation

The reaction mixture (5 mL) consisted of 0.2 mL of 1% bovine albumin, 4.78 mL of phosphate buffered saline (PBS, pH 6.4), and 0.02 mL of extract, and the mixture was mixed, and was incubated in a water bath (37 °C) for 15 min, and then the reaction mixture was heated at 70 °C for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). Phosphate buffer solution was used as the control[12].

% inhibition of hemolysis = [(A1 – A2)/ A1] x 100

Where A1 = absorption of the control sample, and A2 = absorption of the test sample.

Proteinase Inhibitory Activity

The reaction solution (2 mL) consisted of 0.06 mg trypsin, 1 mL of 20 mM Tris-Hcl buffer (pH 7.4), and 1 mL test sample (0.02 mL extract 0.980 mL ethanol). The solution was incubated (37 °C for 5 min), and then 1 mL of 0.8% (w/v) casein was added,and the mixture was further incubated for an additional 20 min. At the end of incubation, 2 mL of 70% perchloric acid was added to terminate the reaction. The mixture was centrifuged, and the absorbance of the supernatant was measured at 210 nm against buffer as the blank. Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula[13].

% inhibition of hemolysis = [(A1 – A2)/ A1] x 100

Where A1 = absorption of the control sample, and A2 = absorption of the test sample.

RESULT:

Phytochemical screening

The ethanolic extract of the peels of *Ipomoea batatas* was evaluated for the presence of different phytochemicals like alkaloid, glycoside, carbohydrate, tannin, phenols etc.

The study revealed the significant presence of the secondary metabolites like alkaloid, carbohydrate, tannin, reducing sugar, phenolic compounds in the peel extract, however saponin was absent. [Table 1]

TABLE 1: IDENTIFIED SECONDARY METABOLITES IN PEELS OF *IPOMOEA BATATAS*

Phytochemicals / Secondary Metabolites	Result
Alkaloids	(+)
Phenol	(+)
Reducing sugar	(+)
Saponins	(-)
Tannins	(+)
Carbohydrates	(+)

Antioxidant Study

Total Phenolic Content:

Total phenolic content (mg/ml) concentration was calculated in terms of Gallic acid equivalent using standard curve equation of Gallic acid [Fig 2]

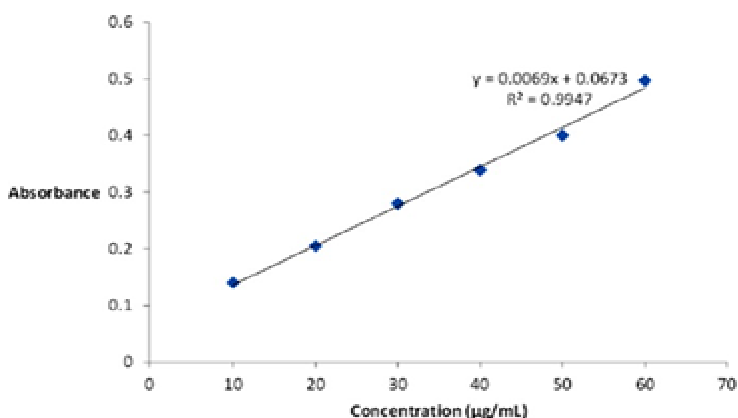

FIG 2: STANDARD CURVE OF GALLIC ACID FOR TOTAL PHENOLIC CONTENT STUDY
Y= 0.0069X + 0.0673, X = (Y-0.0673)/0.0069

Total phenolic content in terms of Gallic acid is 41.11 µg/ml.

Total flavonoid content:

Total flavonoid content (mg/ml) concentration was calculated in terms quercetin equivalent using standard curve equation of quercetin [Fig 3]

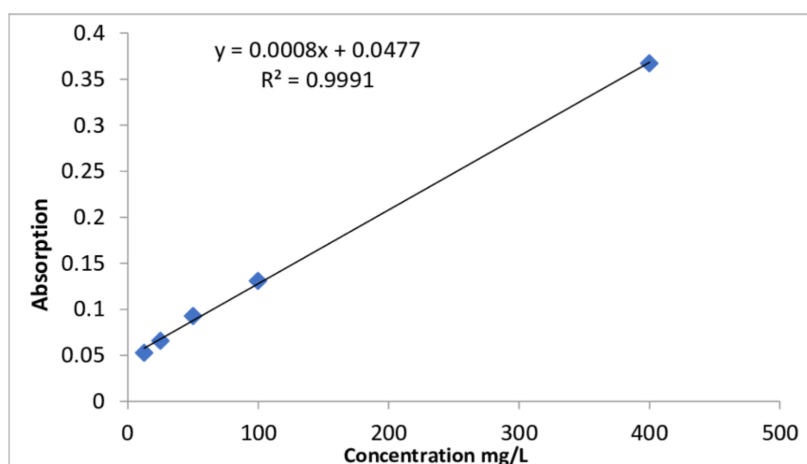

FIG 3: STANDARD CURVE OF QUERCETIN FOR TOTAL FLAVONOID CONTENT STUDY

Y= 0.0008X+ 0.0477, X = (Y-0.0477)/0.0008

Total flavonoid content in terms of Quercetin is 43.90 µg/ml

Anti-microbial study:

Minimum Inhibitory Concentration (MIC) Study:

The study has identified the minimum concentration of the extract required to inhibit the growth of the microorganism [Table 2 & 3]

Zone of Inhibition Study

The zone of inhibition for *Pseudomonus aeruginosa* was 1.7, 1.8, 1.9 and 2.73 cm for the concentration 25, 50, 75, 100 mg/ml of extract respectively [Fig 4]

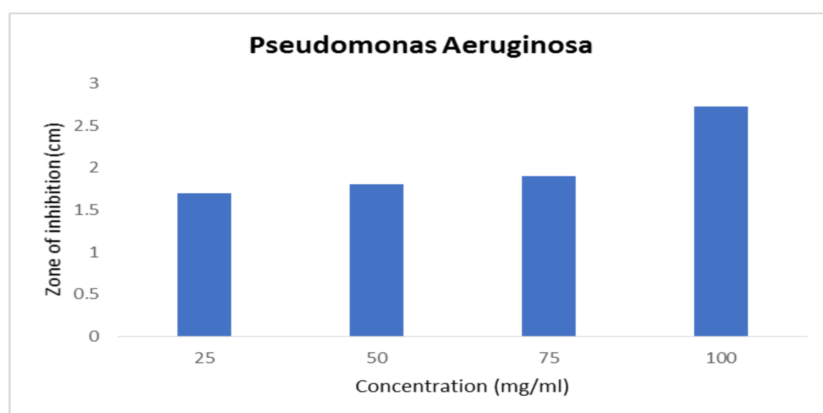

FIG 4 : ZONE OF INHIBITION STUDY OF PSEUDOMONAS AERUGINOSA

Zone of Inhibition Study of Bacteria (*Staphylococcus aureus*)

At all concentration (25, 50, 75,100 mg/ml) of the study extract, it has shown full bacterial growth inhibition.

The zone of inhibition for *Aspergilus niger* was 1.7, 2.3 and 2.8 cm for the concentration 50, 75, 100 mg/ml of extract respectively [Fig 5,6]

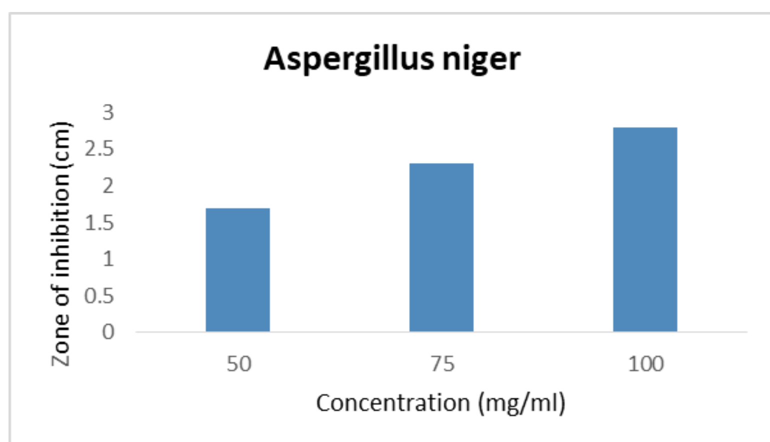

FIG 5: ZONE OF INHIBITION STUDY OF FUNGUS (*ASPERGILLUS NIGER*)

FIG 6: ZONE OF INHIBITION STUDY

TABLE 2: MICROBIAL GROWTH AT VARIOUS CONCENTRATION OF PEEL EXTRACT

Microorganism	Concentration(mg/ml)				
	15	**25**	**50**	**75**	**100**
Staphylococcus aureus	+	-	-	-	-
Pseudomonas aeruginosa	+	–	-	-	-
Aspergillus niger	+	+	–	–	–

(+): Bacterial Growth Visible **(-): Bacterial Growth Inhibited**

TABLE 3: MINIMUM INHIBITORY CONCENTRATION (MIC) OF PEELS OF *IPOMEA BATATAS*

Microorganisms	Minimum Inhibitory Concentration
Staphylococcus aurous	25mg/ml
Pseudomonas aeruginosa	25mg/ml
Aspergillus niger	50mg/ml

Anti Inflammatory Study

From the UV visible spectroscopy statistical analysis through regression equation showed that good correlation exists between the concentration of extract and % Inhibition, with R2= 0.9881 & IC_{50} (Inhibitory concentration) is **213 µg/ml** [Fig 7]

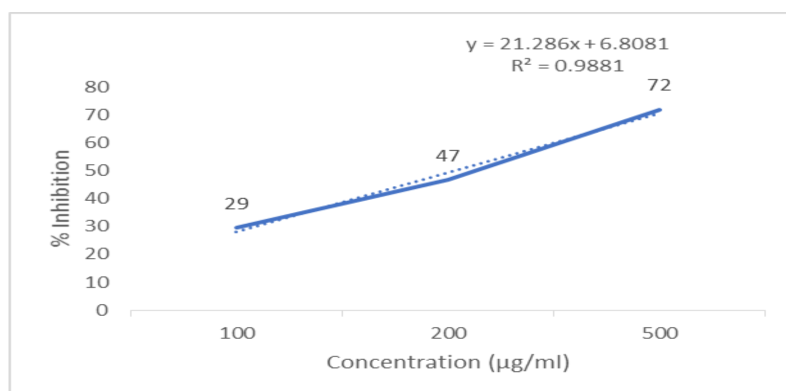

FIG 7: IN VITRO ANTI-INFLAMMATORY STUDY OF SWEET POTATO PEEL EXTRACT BY HYPOTONICITY INDUCED HEMOLYSIS METHOD

From the UV visible spectroscopy statistical analysis through regression equation showed that good correlation exists between the concentration of extract and % Inhibition, with R2= 0.9405 and IC_{50} (Inhibitory concentration) is **232µg/ml.** [Fig 8]

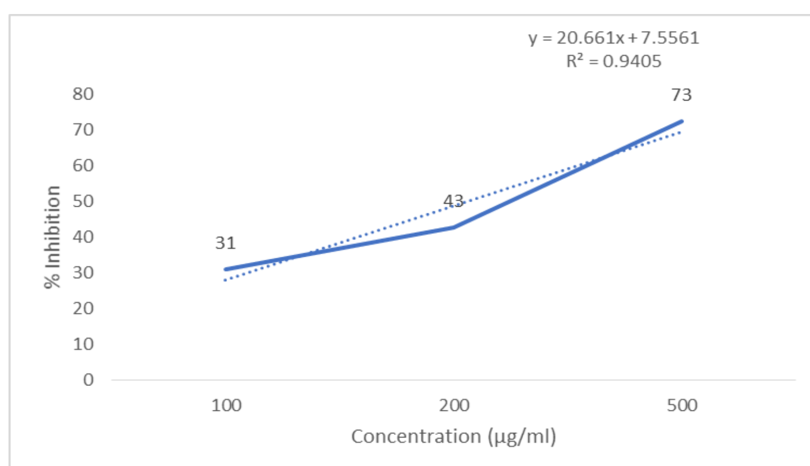

FIG 8: IN-VITRO ANTI INFLAMMATORY STUDY OF SWEET POTATO PEEL EXTRACT BY PROTEIN DENATURATION METHOD

From the UV visible spectroscopy statistical analysis through regression equation showed that good correlation exists between the concentration of extract and % Inhibition, with R2= 0.9771 and IC_{50} (Inhibitory concentration) is **208µg/ml.** [Fig 9]

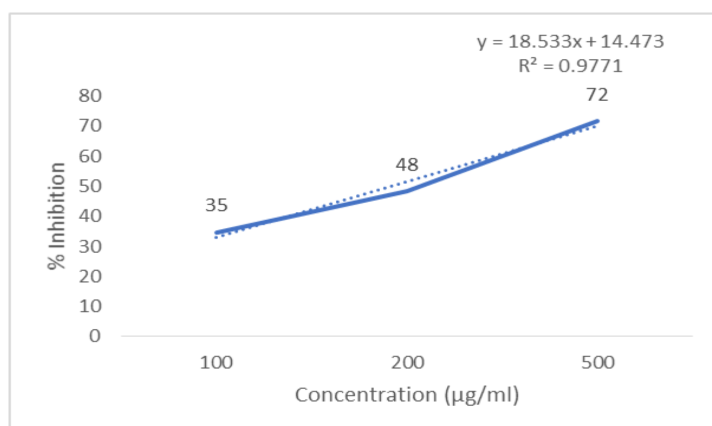

FIG 9: IN VITRO ANTI-INFLAMMATORY STUDY OF SWEET POTATO PEEL EXTRACT BY PROTEINASE INHIBITORY ACTIVITY METHOD

From the UV visible spectroscopy statistical analysis through regression equation showed that good correlation exists between the concentration of extract and % Inhibition, with R2= 0.9362 and IC_{50} (Inhibitory concentration) is **378µg/ml** [Fig 10]

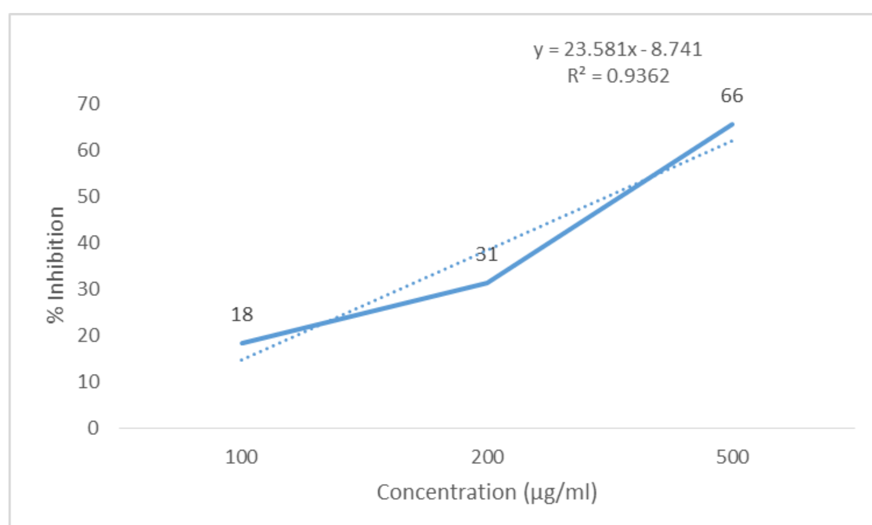

FIG 10: IN VITRO ANTI-INFLAMMATORY STUDY OF SWEET POTATO PEEL EXTRACT BY HEAT INDUCED HAEMOLYSIS METHOD

DISCUSSION AND CONCLUSION :

The collected plant samples have been subjected to ethanol extraction by maceration process and then followed by the estimation of yield. The preliminary phytochemical study has revealed that the selected plant product contains secondary phytoconstituents like alkaloids, tannins, phenols, reducing sugars etc. After preliminary investigation several biological analyses has been conducted to evaluate the health benefits like in vitro antioxidant, anti-inflammatory and anti-microbial study against bacteria and fungus. Phenols in the form of phenolic compounds mainly acts as natural antioxidants which is essential to prevent any degenerative diseases[14] Natural antioxidants scavenge the free radicals due to its structural entity and lipid peroxidation[15]

So, it can be correlated in between total phenolic and flavonoid content with anti-inflammatory study. In terms of standard samples the plant sample contain very potent amount of flavonoid and phenolic compound which are 43.90 µg/ml & 41.11 µg/ml respectively. The assessment of in vitro anti-inflammatory properties consists of protein denaturation study, proteinase inhibitory activity, hypotonicity induced haemolysis and heat induced haemolysis. Tissue inflammation can be caused by cell protein denaturation and it can be reduced by indirect inhibition of proteinase enzyme[16]. On the other side, RBC heamolysis can be revealed as important insights into the inflammatory activity. Hence, haemolysis inhibition can minimize the inflammatory response[17] Therefore, the study of haemolysis inhibition has been done inducing hypo-tonicity

and heat. In this study, the IC_{50} value are increasing with the increase of concentration of ethanolic extract of *I. batatas* throughout the mentioned four types of in-vitro anti-inflammatory study.(Fig.7,8,9,10) The IC_{50} value of protein denaturation, proteinase enzyme inhibitory, hypotonicity induced haemolysis and heat induced haemolysis are 232 µg/ml, 208 µg/ml, 213 µg/ml & 378 µg/ml respectively. Anti-microbial study has been performed against antibiotic resistant strain *Staphylococcus Aureus* (Gram Positive) and *Pseudomonas aeruginosa* (Gram negative) followed by anti-fungal activity against *Aspergillus niger*, responsible for various opportunistic infections . To assess the effectiveness of selected plant sample antibiotic resistant bacteria and opportunistic fungus the minimum inhibitory concentration (MIC) and zone of inhibition study has been taken as promising evaluation tool. In this study, the plant extract shows potent inhibition against the microorganisms. The ethanolic extract of *I. batatas* has 25 mg/ml of minimum inhibitory concentration against *S. aureus* and *P. aeruginosa* and 50 mg/ml of minimum inhibitory concentration against *A. niger*. In case of zone of inhibition study it can be explored that the plant extract is having full inhibition against the growth of *S.aureus*. On the other side, the ethanolic extract of the selected plant reveals the potency of inhibitory activity against gram negative bacteria *P. aeruginosa* and fungus, *A. niger* with increasing extract's concentration. (Fig. 4 & 15).

From the above discussion it becomes very much evident that having a potent anti-inflammatory potential against various inducing factors like hypotonicity, heat, protein denaturation, proteinase is extremely helpful to ensure a natural source against various inflammation induced disorders like diabetes, Alzheimer's, rheumatoid arthritis, nephritis, bronchitis etc. As we know antibiotic resistance is developing issues in the present world where antibiotic resistant strain like *Staphylococcus aureous*, *Pseudomonus aeruginosa* responsible for disease like abscesses (boils), furuncles, cellulitis, pneumonia (chest infections) urinary tract infections, wound infections where the extract of peels of Ipomea batatas has shown potential effect thus it can be considered as a natural cure for the above - mentioned diseases. The extract has also shown then anti-fungal effect against *Aspergillus niger* which helps to conclude that it can be a new herbal approach to prevent opportunistic infections.

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