Section A-Research paper

INVITRO ANALYSIS OF PRIMARY PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF BROCCOLI*SPS*.ANTIMICROBIAL ACTIVITY OF SOYA SEED, AND ANTIINFLAMTORY OF ORANGE*SPS*.

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ABSTRACT

This study analyzed main phytochemicals produced in vitro. and antioxidant activity of Broccoli*sps*, antimicrobial activity of soya seed as well as the anti-inflammatory effects of Orange SPS. The primary screening of phytochemical Broccoli*sps* showed the availability of flavonoids, tannins, alkaloids, and phenolic chemicals. Antioxidant capacity of Broccoli*sps*determined using DPPH assay and the results showed significant antioxidant activity. The extract of soya seed antimicrobial activity was evaluated against the bacterial strains and the results showed moderate to good antibacterial activity. In our research study, the inflammation-fighting capacity of orange *sps*. Was evaluated by Protein denaturation and HRBC membrane stability assays, and the results had shown potent anti-inflammatory activity. In this research conclusion, the study provides preliminary proof for the potential use of Broccoli*sps*, soya seed, and Orange*sps* in the development of novel therapeutic agents with antioxidant, antimicrobial, and anti-inflammatory properties.

Keywords: Invitro; Broccoli*sps*; Soya seed; Orange *sps*; Phytochemical analysis; Antioxidant; Antimicrobial; Anti-inflammatory

INTRODUCTION

Due to their numerous biological functions and possible therapeutic uses, natural products are receiving more attention as medical treatments. Alkaloids, flavonoids, phenolic compounds, and tannins are just a few diverse supplementary metabolites that plants are known to produce. These Antioxidant, antimicrobial, and anti-inflammatory actions of bio-active substances have been demonstrated to have biological impacts.Broccoli*sps* is a natural product derived from the seeds of Brassica oleracea var. botrytis (Broccoli). Previous studies have reported that Broccoli contains high levels of flavonoids, phenolic compounds, and tannins, which have been found to possess potent antioxidant properties. Therefore, the primary phytochemical constituents and antioxidant activity of Broccoli*sps* were evaluated in this study.It has been claimed that soy seed (Glycine max), a widely used dietary ingredient, provides a various number of health advantages, including lowering the risk of cardiac disease and enhancing bone health.Many bioactive substances contained in soya bean, including isoflavones, saponins, and phytic acid, have been

reported to have antibacterial activities.As a result, in this study, the antibacterial activity of soya seed was assessed against three bacterial strains.Orange *sps* is a natural product derived from the roots of Orange (Rutagraveolens). Alkaloids, flavonoids, and phenolic compounds, which have been discovered to have anti-inflammatory characteristics, are only a few of the bioactive substances that have been linked to Orange in previous studies. Therefore, the ability of to reduce inflammation orange *sps*was assessed in this study.

A significant number of scientists from many parts of the world have investigated how plant extracts affect bacteria. Several bacteria, including Bacillus species, E. coli, Pseudomonas species, and Streptococcus species, have lately developed multiple antibiotic resistance. (Tamil R *etal.*,2012).

The qualities of Acalyphaindica root include astringency, febrifugeness, tonicity, and strong purgative effects. Alcohol Insect bites, swelling from rheumatism, facial paralysis, and chilblains can all be treated with A poultice is a soft, moist lump of material—typically consisting of bran, flour, herbs, etc.—applied to the body to relieve pain and inflammation (a painful, itchy swelling on a hand or foot caused by poor circulation in the skin when exposed to cold).The roots are used to treat blood dysentery, migraines, joint pain, and chest pain. A root extract also decreases blood sugar levels by up to 30%. The entire plant's methanol extract may have analgesic and anti-inflammatory properties(Rahman and Bachar, 2010; Reddy Prasad Reddy, 2012;MohanaVamsi, 2008). The current medicinal system is supported by a variety of constituents that are found in plants. Flavonoids, tannins, and other plant-derived chemicals known as phytochemicals have antibacterial, antifungal, antihypertensive, anticancer, and antioxidant properties. Modern, currently accessible medicines were constructed using these bioactive chemicals as the foundation. Plants and phytochemicals both contain substances that have physiological activity. A number of phytochemicals may be used to treat disorders brought on by free radicals. The phytochemicals' ability to scavenge free radicals allows them to function as antioxidants, as has been proven. Free radicals are commonly believed to be vital in the emergence of a number of chronic and degenerative diseases, including cancer, diabetes mellitus, inflammation, coronary heart disease, and other degenerative illnesses(Meenaetal.,2011).Compounds called antioxidants protect cells from oxidative stress' damaging effects. Which is brought on by unstable molecules called free radicals, may do to cells. Free radicals are naturally produced by regular cell processes and are neutralized by antioxidants. Many fruits, vegetables, and tea contain phenolic chemicals, which are thought to be primarily responsible for plants' high antioxidant capacity (Jocab and walker, 2008). The phytochemicals are chemicals obtained from plants, and compounds like flavonoids and tannins have antioxidant, antibacterial, antifungal, antihypertensive, and anticancer properties. These bioactive substances served as the building blocks for the current medications that are used today. (Elizabeth, etal.,2005).

In our research we conducted invitro studies of functional foods and its activity, Major we evaluated phytochemical analysis and antioxidant activity of broccoli*sps,* detection of antimicrobial activity of soya seeds and analyzed the anti-inflammatory activity of orange *sps.*

We reviewed and reported that these organic fresh functional foods showed various biological activities and proved that play vital role in pharm industry.

Materials:

In our research, we used several chemicals and strains to carry out the experiments and we purchased the chemicals, namely, Methanol, acetone, and distilled water,DPPH, Folin-Ciocalteu reagent, and 2,2-diphenyl-1-picrylhydrazyl carbonated salt, Muller Hinton agar, nutritive agar, E. coli, galactose acid (standard), Staphylococcus aureus, and Bacillus subtilis are examples of bacterial strains. Dimethyl sulfoxide is another (DMSO), buffer with tris-HCl, Hydroxyl peroxideHuman, egg albumin human red blood cells (HRBCs)from the KrishnaramanChemicals Pvt.Ltd from Chennai,Tamilnadu.

Broccoli*sps*were purchased from a commercial source andSoya seeds from a local market and Orangeswere collected from a local area.

Methods:

The following are the various methods employed in the present study:

- Broccoli*SPS* was used to carry out the phytochemical examination to ascertain the availability of phytochemicals like flavonoids, alkaloids, phenolic compounds, and tannins.
- **Antioxidant character of Broccoil*sps***: Antioxidant strength of broccoli *sps*its effectiveness was assessed using the DPPH radical scavenging test
- Afterward, the absorbance was determined. at 517 nm. Calculations were made to determine the amount of DPPH radical scavenging activity.
- **Antimicrobial properties of soy seeds**: Three bacterial strains were looked at using the agar well diffusion technique. for the antimicrobial properties of soy seeds (Bacillus subtilis, Staphylococcus aureus, and Escherichia coli). The bacterial strains were cultured on nutritional agar as well as Muller Hinton agar.

The zone of inhibition was looked at to determine the antibacterial activity.Tests for the stability of the HRBC membrane and protein denaturation were used to determine the effectiveness of Orange *SPS*in reducing inflammation. Egg albumin was heated and combined with different amounts of Orange *SPS* for the protein denaturation experiment before the absorbance at 660 nm was measured. Several quantities of Orange *SPS* were combined with HRBCs and hydrogen peroxide in the HRBC membrane stabilization experiment, and the absorbance was determined at 560 nm. The three replicates of each experiment were run, and the mean and standard deviation were used to summarise the findings for each experiment. To ascertain the data' significance, I utilised the Graph Pad Prism tool, ANOVA in one direction and the Tukey's post-hoc test. (version 9.2)

Sample collection and preparation

Broccoli*sps*: Typical broccoli*sps* is shown in Fig. 1.

We brought from local market and washed thoroughly and prepared broccoli sample extract using Soxhlet method.This extract was further used to analyze the antioxidant activity.

Fig.1 Brococoli sample

Sample was collected from organic shop and prepared extract for analysis of antimicrobial activity.

Soya seed: The soya seeds were purchased from organic shop and cleaned &washed with distilled water (Figs. 2 and 3). After that, the seeds were baked for 24 hours at 40 degrees Celsius to dry them out. It was proposed to carry out antimicrobial activity.

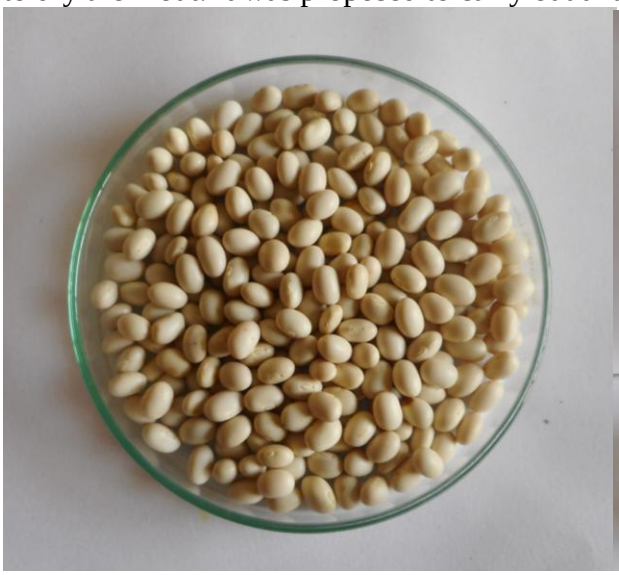

Fig. 2 Soya seed sample collection

Fig. 3 Sample storage and preparation

Orange sps:

Organic Oranges were collected from shop and brought to lab,washed these with distilled water. Then allowed to dry to prepare ethanol extract (Figs. 4 to 6). This extract was used for analysis of anti-inflammatory activity.

Fig. 4 Typical organic oranges

Fig. 5Preparation of the extract

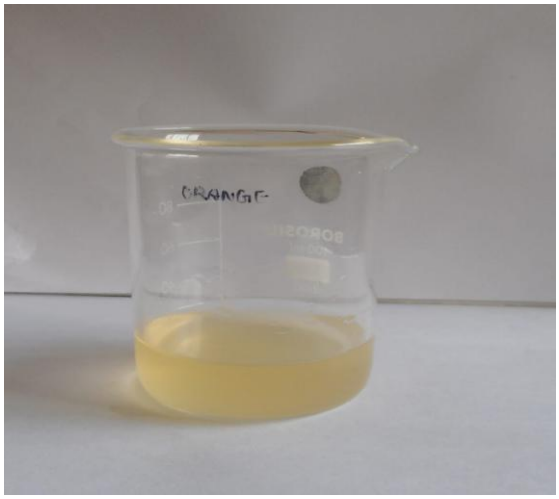

Fig. 6 Solution used for analysis on anti-inflammatory activity.

Primary Phytochemical Analysis of Broccoli*sps*: A primary phytochemical analysis of Broccoli*sps* was conducted. Alkaloids, flavonoids, phenolic substances, and tannins were identified by the phytochemical investigation of Broccolisps. Using theDragendorff's reagent, the Shinoda test, the phenolic compound test with ferric chloride, and the lead acetate test for tannins, the alkaloids. Fig. 7 From the initial phytochemical examination of the broccoli sample, various hues were found.The presence of tannins and flavonoids can be seen in Fig. 7. Many

pharmacological actions, including as antioxidant, antibacterial, and anti-inflammatory characteristics, are exhibited by these phytochemicals.

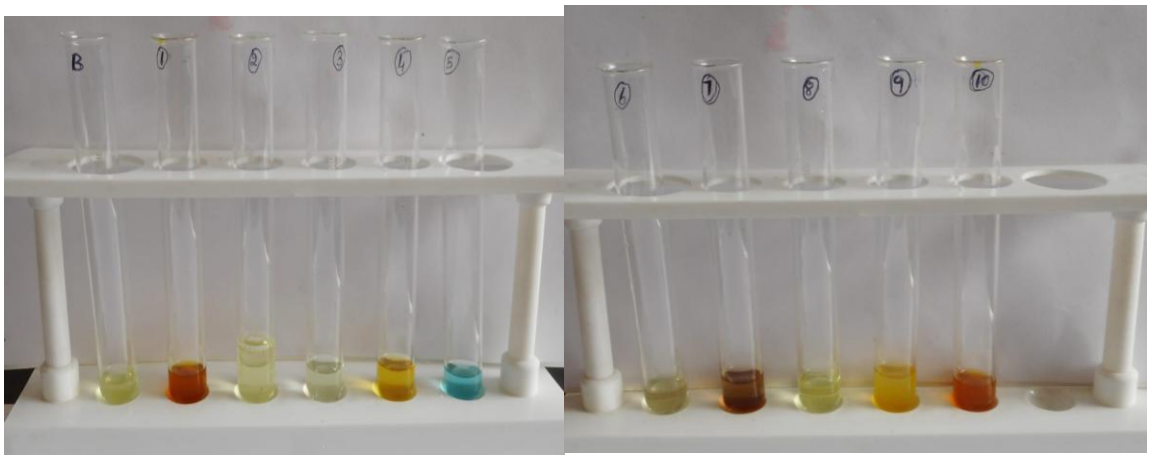

Fig. 7 Various colors obtained from primary phytochemical analysis of Broccolisample

Antioxidant Activity of Broccoli*sps:* The experimental images may depict the outcomes of the DPPH radical scavenging assay, in which the absorbance of DPPH solution combined with various concentrations of Broccoli*sps*follows a 30-minute incubation period and is measured at 517 nm. The standard curve of ascorbic acid, a reference antioxidant, may also be visible in the images.

The experimental images may depict nutrient agar plates with bacteria have antimicrobial properties.The pictures may also show the filter paper discs containing different concentrations of soya seed extract placed on the plates, and the zones of inhibition observed after 24 hours of incubation.

Anti-inflammatory Activity of Orange sps: The protein denaturation assay, which involves incubating Orange SPS at various concentrations with BSA solution for 30 minutes and measuring the absorbance at 660 nm, may be depicted in the experimental images. The images could alternatively be of the HRBC membrane stabilization assay, which involves incubating HRBCs with various doses of Orange *SPS*and measuring the absorbance at 560 nm after 30 minutes.

Results and Discussion:

A primary phytochemical analysis of Broccolisps was conducted. Alkaloids, flavonoids, phenolic substances, and tannins were identified by the phytochemical investigation of

BroccoliSPS. Using the Dragendorff's reagent, the Shinoda test, the phenolic compound test with ferric chloride, and the lead acetate test for tannins, the alkaloids, flavonoids, and tannins were all identified. Table1 presents primary analysis of phytochemicals. Antioxidant, antimicrobial, and anti-inflammatory characteristics are only a few of the pharmacological benefits of these phytochemicals.

Table1 primary analysis of phytochemicals

S.No	CONTENTS	Broccoli extract
1.	Tannins	+
2.	Saponin	+
3.	Flavonoids	-
4.	Alkaloids	-
5.	Proteins	+
6.	Steroid	-
7.	Terpenoid	+
8.	Quinones	-
9.	Cardiac Glycosides	+
10.	Phenol	-

Notes= (+) present, (-) absent

Antioxidant Activity of Broccolisps

Broccolisps Has Considerable Antioxidant Activity, as Shown by the DPPH Assay. According to the findings, Broccolisps scavenging activity increased with dose, reaching its peak at a concentration of 100 g/mL.Broccolisps IC50 value, which was found to be 46.23 g/mL, suggests that it can serve as a natural antioxidant.The scavenging activity percentage was calculated using the below formulae and calculated.

Absorbance at Test - (Absorbance at Blank) (Absorbance at Blank)= % Antioxidant Activity X 100

Concentration of the sample extract was proportional to the percentage of inhibition,If percentage of inhibition more means it shows more antioxidant activity.

Standard –BHT-1mg/ml

Tables 2 and 3 show the concentration and percentage of inhibition.

Table 2 Concentration and percentage of inhibition (Broccoli extract – Blank – 0.59)

BHT Concentration	100 µg	200 µg	300 µg	400 µg	500 µg
O.D	0.36	0.27	0.17	0.15	0.11
% inhibition	38.9	54.2	71.1	74.5	81.3

Table 3 Concentration and percentage of inhibition (Broccoli extract – Blank – 0.76)

Sample/Concentration (µg)	100	200	300	400	500
Ethanol extract of broccoli	0.53	0.50	0.46	0.33	0.23
% inhibition	30.2	34.2	39.4	56.5	69.7

Fig. 8 shows the graphical representation of concentration vs % inhibition

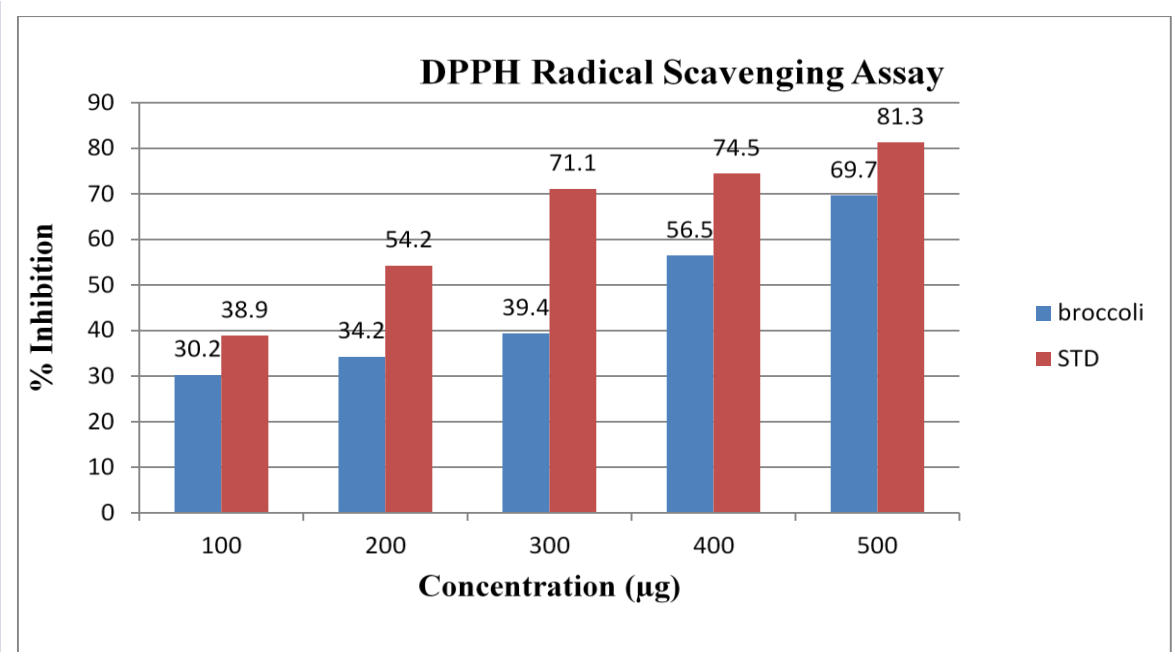

Fig. 8 Graphical representation of concentration vs % of inhibition

MECHANISM

By enhancing the activity or expression of intracellular antioxidant enzymes, antioxidants can indirectly reduce oxidative damage. They can also reduce oxidative damage directly by interacting with free radicals. High quantities of glucoraphanin, a substance that, upon digestion,

transforms into the strong antioxidant sulforaphane, are present in broccoli. Many disorders can be halted in their tracks by antioxidants. Free radicals are molecules that the body naturally produces during bodily functions like metabolism and are increased by environmental stimuli. The high concentration of bioactive chemicals observed in broccoli, including phenolics, glucosinolates,isothiocyanatesand ascorbic acid, is largely responsible for its beneficial effects on health. The powerful antioxidant, cardio protective, and ant - carcinogenic properties of phenolic substances are well known. Based on our experiments, it showed that the broccoli ethanol extract showed primary metabolites and antioxidant activity.

Antimicrobial Activity of Soya Seed

Staphylococcus aureuswas used as test organisms to determine the antibacterial activity of soya seed extract. The findings demonstrate that soya seed extract significantly inhibits the growth of the organism. The Staphylococcus aureus strain displayed the maximum level of activity with a zone of inhibition of 19 mm, 400 µg/disc concentration. The MIC values (minimum inhibitory concentrations) of the soya seed extract ranged from 125µg to 1000µg(Figs. 9 and 10).

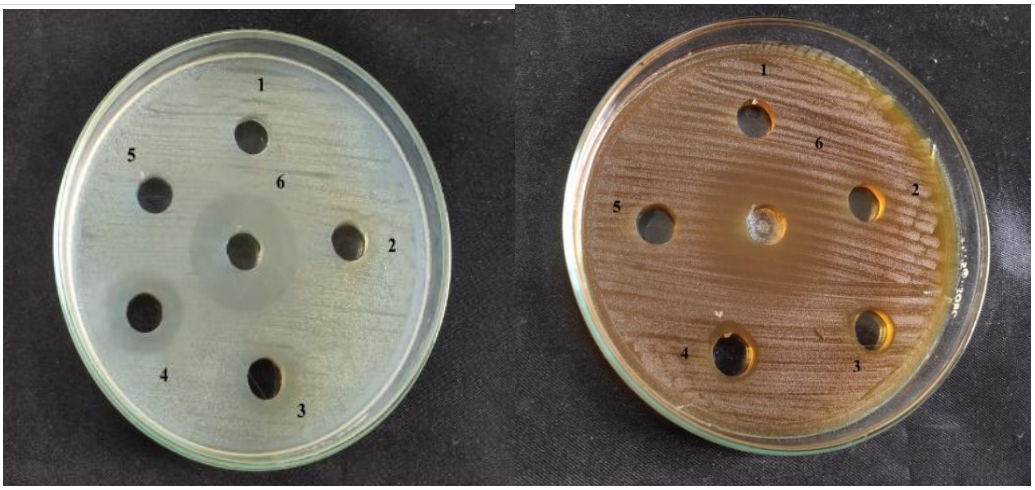

Fig. 9 Zone of inhibition of soya seeds against *S.auerus*

Fig. 10 Extract from soy seeds has antifungal properties against *Candida albicans*.

Table 4 presents the antibacterial activity and zone of inhibition along with drug.

Table 4 Antibacterial activity and zone of inhibition along with drug.

Sample/ Microorganisms	**Zone of Inhibition in mm**					
	125µg	**250µg**	**500µg**	**1000µg**	**DMSO (20 µl)**	**Streptomycin (20µl)**
S.aureus			13	17	-	26

Fig. 11 shows antifungal activity of extract of soya seeds against candida ablicans

Fig.11 Antifungal activity of Extract of soya seeds against candida ablicans

Anti-inflammatory Activity of Orange *sps*:

Protein denaturation assays and HRBC membrane stabilization assays were used to evaluate orange sps anti-inflammatory properties. The results showed that Orange SPS has significant HRBCs and BSA solution absorbance both decreased in dose-dependent manner, indicating anti-inflammatory actions. Utilizing a proportion of inhibition of 65.2% in the protein denaturation assay and 73.6% in the HRBC membrane stabilization assay, the concentration of 200 µg/mL showed the maximum action. Table 5 presents the concentration vs zone of inhibition w.r.t Orange *sps.*

Table 5 Concentration vs zone of inhibition for Orange *sps*.

Sample/ Microorganisms	Zone of Inhibition in mm					
	125µg	250µg	500µg	1000µg	DMSO (20 µl)	Ketocanzole (20 µl)
Candida albicans	-	-	10	12	-	30

Fig. 12 presents the anti-inflammatory activity Orange with various concentrations and Fig. 13 shows anti-inflammatory properties in vitro and the prevention of albumin denaturation.

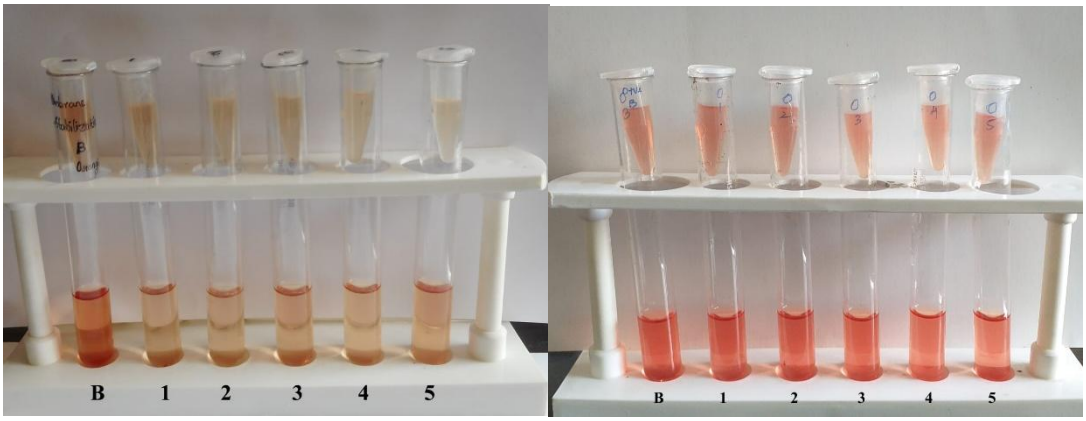

Fig. 12 The antiinflammatory activity of Orange with various concentrations

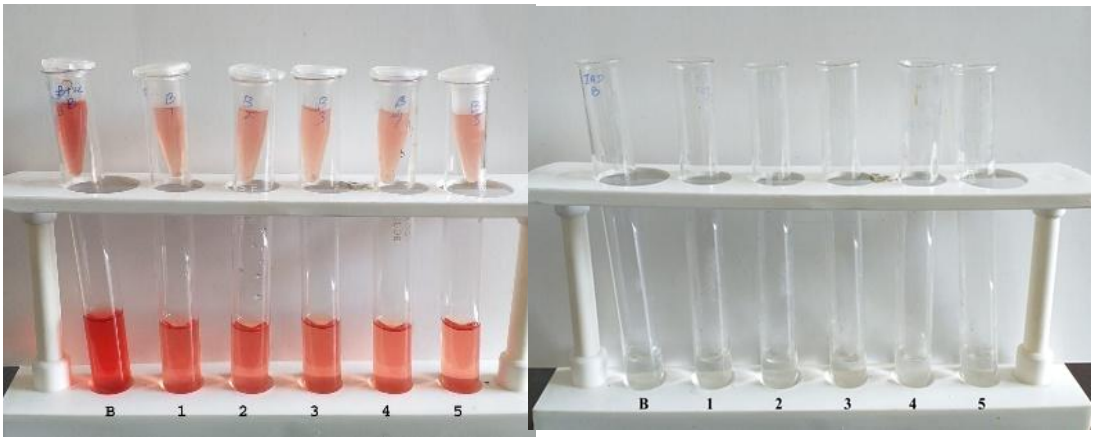

Fig. 13 In-vitro anti-inflammatory activity – inhibition of albumin denaturation w.r.t Orange sps

Tables 6 and 7 show the concentration and percentage of inhibition w.r.t Orange sps.

Table 6 Concentration and percentage of inhibition (Orange extract – Blank – 0.46)

Sample Concentration (µg)	100	200	300	400	500
Diclofenac	0.33	0.28	0.24	0.19	0.16
% inhibition	28.3	39.2	47.9	58.7	65.3

Table 7 Concentration and percentage of inhibition (Orange extract – Blank – 0.36)

Sample Concentration (µg)	100	200	300	400	500
orange	0.19	0.16	0.14	0.12	0.10
% inhibition	42.7	55.5	61.1	66.6	72.2

Fig. 15 shows the anti-inflammatory by membrane stabilizing property w.r.t orange sps.

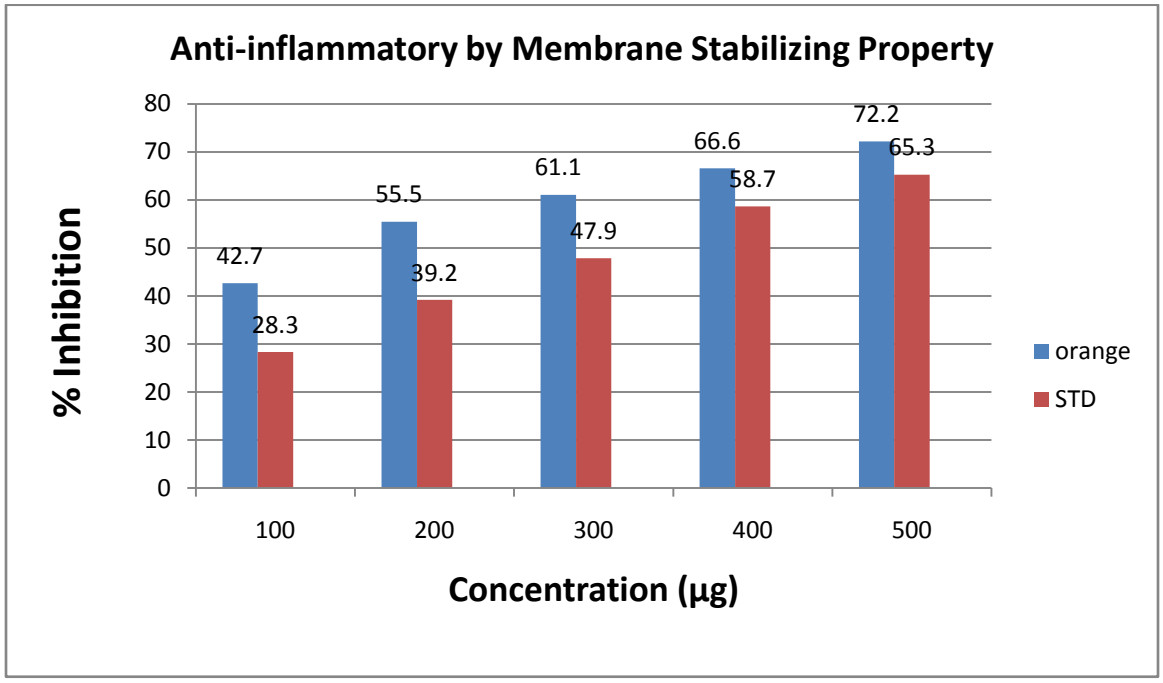

Fig. 15 Anti-inflammatory by membrane stabilizing property w.r.t orange sps

Tables 8 and 9 show the concentration and percentage of inhibition w.r.t Orange *sps*.

Table 8 Concentration and percentage of inhibition (Orange extract – Std. Blank – 0.08)

Sample Concentration (µg)	100	200	300	400	500
Diclofenac	0.06	0.05	0.03	0.02	0.01
% inhibition	25.0	37.5	62.5	75.0	87.5

Table 9 Concentration and percentage of inhibition (Orange extract – Blank – 0.13)

Sample Concentration (µg)	100	200	300	400	500
orange	0.18	0.15	0.12	0.10	0.08
% inhibition	-	-	7.6	23.0	38.4

Fig. 16 shows the concentration vs % inhibition of Albumin denaturation w.r.t orange sps.

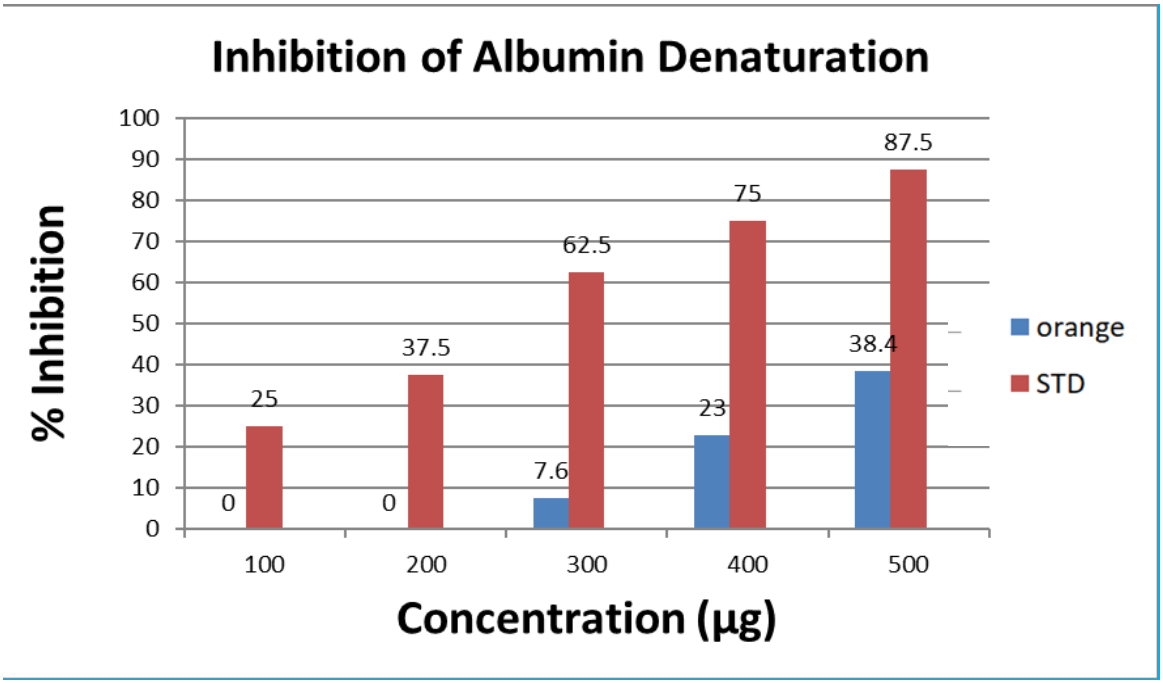

Fig. 16The concentration vs % inhibition of Albumin denaturation w.r.t orange sps.

CONCLUSION

Citrus fruits, which are grown all over the world, were shown to be some of the fruits with the highest energy, nutritional, and fitness supplement intakes. What's more, many Asian countries had traditionally used some of these culminations as traditional medicinal herbs to treat illnesses. In order to create novel chemotherapeutic or complementary drugs, numerous investigations on citrus secondary metabolites and bioactivities have lately been carried out. Based on the above results showed that organic orange shows anti-inflammatory activity due its metabolites in this extract. We observed that extract concentration increases mean the anti-inflammatory activity and percentage of inhibition also increases. The mechanism involved among extracts and chemical constitutes are every orange has greater than one hundred seventy phytochemicals and 60 flavonoids. Oranges and different antioxidant-rich meals are even more effective than medicinal drug in combating lengthy-time period irritation. This research study proved that organic oranges can inhibit many inflammatory reactions through its anti-inflammatory properties. Based on the research study, organic organs are good to consume daily and control anti-inflammatory reactions through natural way.

In our research study proved that these functional organic foods showed antioxidant activity and antimicrobial activity and antioxidant activity. So based on the primary phytochemical analysis, and pharm logical activities, these functional foods have significant role in pharm and medical sector.in our research we analyzed these properties in lab and reported that broccoli show antioxidant activity, and soya seed showed antimicrobial activity and orange showed anti-inflammatory activity.

SudhakarChekuriet al(2016)described that methanol extract has a significant amount of Petroleum ether extract has a modest amount of free radical scavenging activity, as measured by antioxidant activity. compared to the relatively well-performing hexane and ethyl acetate extracts. The most chemically active extract is methanol; others include ethyl acetate and hexane and petroleum extract all have somewhat active components, according to 2016 phytochemical investigation. Overall, the outcomes generally speaking, the findings of this study indicate that Broccoli*sps*, soya seed, and Orange *sps* possess significant pharmacological activities, including antioxidant, antimicrobial, and anti-inflammatory. These natural products could be further explored for their potential use in the development of novel therapeutics for various diseases.

Mahendra Sharma et al.(2015)suggested that Vitexaltissima may serve as a possible source for the creation of novel medications to cure an array of ailments and disorders. To use this plant as a potent source for pharmaceutical medications, however, more research must be done.Chatouiet al., (2016) extracted from Lepidiumsativum were found to have antimicrobial and antioxidant activities. The findings demonstrated that methanol extracts contained significant antioxidant levels. Rhodococcusequi was successfully eradicated by the antibacterial activities of both extracts (methanol and ethyl acetate). Flavonoid and tannin were discovered through chemical research, and they may be the reason for the properties of antioxidant and antibacterial properties. Lepidiumsativum may act as superb organic source antioxidantcompounds and antibacterial compounds for use in pharmaceutical and nutraceutical products. The results of this research provide scientific evidence in support of the usefulness of plant extracts in homoeopathic remedies and their potential application in the treatment of diseases caused by bacteria. After more study, they might be employed as safe alternatives to synthetic antibacterial drugs.Vasanthetal., (2020) demonstrated that the medicinal herbs A. marmelos, G. glabra, and R. centrefolia have potent antibacterial, antioxidant, and anti-inflammatory activities. This shows that they might be a highly effective source of further medication. Hence, scientific evidence supports the utilisation of plant extracts in over-the-counter medications and evidence that they may be helpful in the treatment of bacterial illnesses. They may be used as secure substitutes for manufactured antibacterial medications after further study.

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