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EXTRACTION OF FLAVANOIDS FROM GINGER, GARLIC, BLACK PEPPER AND HIBISCUS AND EVALUATION OF ITS ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Flavonoids are antioxidant molecules which possess antibacterial and antifungal properties. They are potential reducing agents that provide protection from UV radiation and oxidative damage. Spices like ginger, garlic and pepper are indispensable for the preparation of our daily food and are reported to possess compounds, which have varied beneficial biological effects. The present study evaluates the antioxidant and the antimicrobial activity of the flavonoids extracted from *Hibiscus rosa-sinensis* (hibiscus), *Piper nigrum* (black pepper), *Zingiber officinale* (ginger) and *Allium sativum* (garlic) against *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa)* and *Aspergillus niger* (A. niger). Flavonoids were extracted using 95% ethanol as a solvent. The presence of flavonoids in the ethanolic plant extracts was detected using NaOH and HCl and the flavonoid estimation was carried out using the AlCl3 method. Antioxidant activity of all the extracts was determined using phosphomolybdenum assay. Results obtained indicated that flavonoids extracted from all the four plant materials were effective against *S. aureus and A. niger*. Flavonoids obtained from hibiscus, ginger and garlic could inhibit the growth of *E. coli* whereas the one's obtained from black pepper proved to be ineffective. The growth of *P. aeruginosa* was not inhibited by any of the extracted flavonoids thus rendering them ineffective against it. Thus, in the light of several factors such as increasing drug resistance, allergies towards synthetic compounds, side effects, etc., the present study encourages to use flavonoids as an alternative or supplementary drug and also supports its use in traditional medicines, various skin care products and other medicinal formulations with further investigation.

Key words: Flavonoids, antioxidant, antimicrobial activity, phosphomolybdenum, $AlCl_3$.

INTRODUCTION:

Herbs and spices are very important and useful as therapeutic agents against many pathological infections (Gull et al., 2012). The spices have a unique aroma and flavour, which are derived from compounds known as phytochemicals or secondary metabolites (Avato et al., 2002; Melvin et al., 2009). Phytochemicals are antimicrobial substances present in the spices which are capable of attracting benefits and repel harmful organisms (Melvin et al., 2009). Numerous classes of phytochemicals including the isoflavones, anthocyanins and flavonoids are found associated with the spices (Chang, 1988; Melvin et al., 2009).

Increasing multidrug resistance of pathogens forces to find alternative compounds for treatment of infectious diseases (Gull et al., 2012). At present, the search for antifungal and antibacterial drugs has received attention mainly as a result of considerable drawbacks in the use of major antibiotics. These include those of limited antimicrobial spectrum that will cause serious side effects and high incidence of resistance in bacteria (Olayemi and Opaleye, 1999). Antioxidants are substances that are capable of neutralizing the harmful effects of the reactive oxygen species (ROS) (Momoh et al., 2016). Antioxidants play a vital role in free radical scavenging and chain breaking of oxidation reactions, both in vivo and in vitro. Free radicals possess free unpaired electrons, making them highly unstable and can extract electrons from other molecules to attain stability causing them damage. Among the potential uses of antioxidants, some are: prevention of diseases related to oxidative stress in humans and prevention of oxidative reactions in pharmaceuticals, cosmetic products, and food (Muhammad Abdul Qadir, et al., 2017).

The antioxidant effect of plants is mainly due to phenolic components like flavonoids (Momoh et al., 2016).

Utilization of synthetic antioxidants, i.e. citric acid, propyl gallate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) in foods can have various adverse effects. For instance, these synthetic antioxidants have carcinogenic effect in living systems and can also induce DNA damage. Consequently, there is an increase interest in finding natural antioxidant agents capable of scavenging free radicals and hindering oxidative rancidity of lipids, in this way, protecting living organisms from diseases.

Vegetables, grains, spices, and fruits contain a huge variety of bioactive phytochemicals. The antioxidants which

are derived from plants may function as free radical scavengers, metal-ion chelators, and reducing agents. Many plants possess antioxidant properties. Antioxidants extracted from them, either in the form of raw extracts or as their chemical constituents are very effective to stop the destructive processes caused by oxidative stress (Muhammad Abdul Qadir, et al., 2017).

Flavonoids are antioxidant molecules that are naturally found in plants and fruits. They are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation, and floral pigmentation. They are rich in antioxidant activity and can help your body to ward off every day toxins. They also have antibacterial and antifungal activity against pathogenic microbes (Muhammad Abdul Qadir et al., 2017). Including generous amount of flavonoids in your diet is a great way to help your body stay healthy and potentially decrease the risk of some chronic health conditions. They help regulate cellular activity and fight off free radicals that cause oxidative stress.

Types of flavonoids are flavanols, flavan-3-ols, flavones, flavanones, isoflavones and anthocyanins. They are present in different concentrations in different plants, thus rendering different antimicrobial properties to various plants parts. Inflammation is one of body's immune response. Allergens, germs, toxins, and other irritants can trigger inflammation that results in uncomfortable symptoms. Flavonoids help to dismiss the inflammatory reaction so that the symptoms are reduced.

The aim of the present research was to extract flavanoids and evaluate their antioxidant, antibacterial, and antifungal activities and to estimate the total flavonoid content of *Allium sativum* (garlic), *Zingiber officinale* (ginger), *Piper nigrum* (pepper) and *Hibiscus rosa-sinensis* (hibiscus) extracts.

Ginger (*Zingiber officinale*) is one of the most commonly consumed dietary condiments in the world. The main active phytochemicals present in ginger are gingerols, shogaols and paradols, and they have strong antioxidant and chemo-preventive properties. Ginger extracts have been extensively studied for a broad range of biological activities including anti-bacterial, anti-tumour, anti-fungal, anti-thrombotic, anti-allergic, and other

beneficial activities (Virendra V. Panpatil, et al., 2013).

Garlic (*Allium sativum L*.) is one of the most important plants in the world because of the important substances which it contains that provides protective and healing effect. Many studies have shown that garlic can help prevent cold, cough, flu, pulmonary diseases, and has antibacterial, antifungal, anti-carcinogenic, anti-mutagenic and antioxidant properties. Garlic contains antioxidants that support the body´s defence mechanism against oxidative damage (Ján KOVAROVIČ, et al., 2019). All these properties of garlic are attributed to different phytochemicals present in it. The most important chemical compound of garlic which was thought to be responsible for antimicrobial activity is the organo-sulphur compound including allicin (Hovana, et al., 2011). Garlic has been found to exhibit antibacterial activity against a wide range of Gram negative and Gram- positive bacteria (Chehregani et al., 2007; Pundir; Jain,2010; Bakht et al.,2011; Bakhshi et al.,2012) including multidrug-resistant strains (Ham et al., 2010; Gull et al., 2012).

Pepper (*Piper nigrum L*.,) native of south India is popularly known as “king of spices”. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper. Pepper is included in the prescriptions of many Ayurvedic and other traditional medicinal systems. Pepper is also used in folk medicine as aphrodisiac, carminative, stomachic, antiseptic diuretic and for the treatment of cough, rheumatoid arthritis, peripheral neuropathy due to presence of volatile compounds, tannins, phenols and alkaloids, flavonoids, other unknown substances (S.K. Shiva Rani, et al.,2013).

Many reports are available wherein flowers or their extracts have been shown to exhibit rich antioxidant and anti-microbial properties (Shyu et al., 2009; Jo et al., 2012; Voonet al., 2012). Previous studies have indicated Hibiscus (*H. rosa-sinensis*) to possess bioactive properties and is recommended to be used as an herbal alternative to cure diseases (Obi et al., 1998). Hibiscus is reported as one of the most important medicinal plants that have been widely used for treating heart and nerve diseases. Many chemical constituents such as flavonoid, cyanide, thiamine, riboflavin, niacin, ascorbic acid, etc., have been isolated from this plant. (Ruban P, et al., 2012). Alcoholic extractions of the calyces have established to possess antimicrobial activity against each of *Staphylococcus aureus*, *Escherichia coli.* (Azhar A. Zarkani, 2016).

With the focus to find an alternative for synthetic drugs and antioxidants found in food items, natural substances having anti-oxidative, anti-carcinogenic, antimicrobial properties need to be further studied. It was found necessary to see the antioxidant and antimicrobial activity of garlic, ginger, pepper and hibiscus. Therefore, this study was planned to extract flavonoids and estimate its content in garlic, ginger, pepper and hibiscus and evaluate its antimicrobial and antioxidant activity against pathogens like, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger*.

MATERIAL AND METHODS

Sample Collection

The sources used in the study for the isolation of *E. coli* and *S. aureus* were the contaminated water sample and the soil sample respectively; collected from Vasai, Maharashtra. For the isolation of the fungus *Aspergillus niger*, a piece of bread was damped with water and wrapped in a polythene bag and was maintained at room temperature for 3 days. The micro-organism *Pseudomonas aeruginosa* was graciously shared by Dhevang Rokkla, et al.

Isolation of Micro-Organisms

Serial dilution of the water and soil samples was carried out and 0.1 ml the last three dilutions were inoculated on the sterile nutrient agar plates. The plates were then incubated at 37^0C for 24hrs. After incubation, depending on the morphological characteristics, appropriate colonies were selected, sub-cultured and stored for further studies and subjected for identification tests. The fungal mold obtained on the surface of the bread was transferred on the sterile SDA plate with the help of sterile forceps. The plate was incubated at 37^0C for 72hrs and after incubation was subjected for further tests.

Identification and Characterization of the Isolates

For identification of the bacterial cultures, the isolates were subjected to gram staining and observed under the microscope. Depending on the morphological characteristics, the bacteria were identified as *E. coli* and *S. aureus.* For further confirmation, these bacteria were streaked on selective medias i.e. Mac Conkey and Salt Mannitol Agar (SMA) respectively and biochemical tests such as Indole test; Methyl red (MR) test; Voges-Proskauer (VP) test, Catalase test, Coagulase test, Urease test, Triple sugar iron(TSI), Citrate test and Sugar fermentation tests were carried out as outlined in Bergey's Manual of Systematic Bacteriology.

For the identification of the fungal culture, fungal staining was carried out using lacto-phenol cotton blue stain and was observed under the microscope.

Preparation of Plant Material

The spices used in the study; ginger, garlic and black pepper were collected from the local market and the shrub hibiscus was collected from the garden in Vasai. All the four plant materials were washed with distilled water and allowed to completely dry in the presence of sunlight for a week. They were crushed and ground separately to obtain fine powder. The powder was stored for further use.

Isolation of Flavonoids

For the isolation of free flavonoids, a high alcoholic (ethanol) concentration of about 95% was applied. 30g of each plant material was weighed and transferred to different flasks. To these flasks 60ml of ethanol was added and a ratio of 1:2 was maintained throughout. The flasks were sealed with a cotton plug, wrapped with an aluminum foil and placed on the rotary shaker for 24hrs. After 24hrs, the plant extracts were filtered out using Whatman filter paper no. 1. Ethanol present in the filtrate was evaporated in the hot air oven at 40^0C for about 2 days. The solution obtained thereafter was stored in the dark at room temperature for further use.

Preparation of Inoculum

Bacterial culture suspensions were prepared by inoculating bacterial colonies in 1ml sterile saline (0.85% NaCl v/v). The O.D. of all the culture suspensions was read at 540nm and maintained at 0.2. Fungal hyphae of *Aspergillus niger* were inoculated in 1ml sterile Sabouraud broth using sterile forceps.

Determination of Antimicrobial Activity

The antimicrobial activity of the free flavonoids extracted from garlic, ginger, black pepper and hibiscus was tested against *E. coli, S. aureus, P. aeruginosa* and *A. niger* using agar well method. 1ml of each bacterial culture suspension and 1 ml of inoculated Sabouraud broth were transferred into four different molten sterile Muller Hinton agar butts (20ml) and poured in sterile Petri plates. The agar was then allowed to solidify. With the help of a cork borer, four wells were made in the agar. Using a micropipette, 50µl of each plant (flavonoid) extract was loaded into different wells and the plates were incubated at 37^0C for 24hrs. Positive control plates for *E. coli, S. aureus* and *P. aeruginosa* were also maintained. Sterile conditions were maintained throughout.

Test for Detecting Flavonoids

The presence of flavonoids was detected using dilute NaOH solution. 1ml of each plant extract was transferred in four different test tubes and 1-2 drops of dilute NaOH solution was added. An intense yellow colour appeared in the test tubes, which turned colourless on addition of dilute HCL. This indicated the presence of flavonoids.

Determination of Antioxidant Activity

The total antioxidant capacity of the samples was carried out by the phosphomolybdenum method. This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotomatically. Ascorbic acid was used as a standard and different concentrations (10, 20, 30…100) of the same were prepared using ethanol. 0.5ml of each plant extract was transferred in 4 different test tubes and 0.5 ml of each ascorbic acid concentration was transferred in 10 different test tubes. To these tubes 4.5 ml of phosphomolybdenum reagent was added and vortexed. The tubes were then place in a hot water bath for 90mins and on cooling down, the O.D. was measured at 600nm and a standard graph was plotted.

Estimation of Flavonoids Using $AlCl_3$ Method

Estimation of flavonoids was carried out using the $AlCl_3$ method. $AlCl_3$ forms acid labile complex with the C-4 keto groups and the C-3 or C-5 hydroxyl groups of the flavonoids. Quercetin dihydrate was used as a standard and different concentrations (10, 20, 30…., 100) of the same were prepared using ethanol. 0.5ml of each plant extract was transferred in 4 different test tubes and 0.5 ml of each quercetin concentration was transferred in 10 different test tubes. Next 2ml of ethanol, 0.5ml of AlCl3 and 0.5ml of sodium acetate were added in all the test tubes. The tubes were vortexed and stored in the dark for 40 – 45mins and the O.D. was measured at 420nm and then a standard graph was plotted.

RESULTS

Identification of *Escherichia coli*:

Creamy white colonies of size 3mm - 4mm were observed on the nutrient agar, which were then subjected to Gram staining that indicated Gram negative rods. Presuming the micro-organism as *E.coli,* it was further inoculated on Mac Conkey media, which showed the presence of round, medium sized, pink colonies. The isolate was confirmed by subjecting it through a set of

biochemical tests such as sugar fermentation tests, catalase test, oxidase test, and IMViC tests. Based on the Bergey's Manual of Systemic Bacteriology, the isolate was identified as *E. coli*.

Table 1: Biochemical tests for *E. coli*

Test	**Result for 1st isolate (*E. coli*)**	**Sugar Fermentation test**	**Result for 1st isolate (*E. coli*)**
Indole	+	Glucose	+
Methyl red	+	Sucrose	+
Voges-Proskauer	-	Maltose	+
Citrate	-	Xylose	+
Catalase	+	Lactose	+
Urease	-	Mannitol	+
TSI	+		

KEY: + = Positive - = Negative

Identification of *Staphylococcus aureus*:

Growth of golden-yellow colour, convex colonies of size 2mm – 3mm were observed on the nutrient agar, which when subjected to Gram staining indicated the presence of Gram positive cocci. Speculating the isolate as *S. aureus*, it was further inoculated on the Salt Mannitol Agar, which showed the growth of round, small sized, bright yellow colonies. Also a gradual change in the colour of the media from red to yellow was observed. The isolate was then subjected to a set of biochemical tests: sugar fermentation tests, catalase test, oxidase test, and IMViC tests. Based on the Bergey's Manual of Systemic Bacteriology, the isolate was identified as *S. aureus*.

Table 2: Biochemical tests for *S. aureus*

Test	Result for 2nd isolate (*S. aureus*)	Sugar Fermentation Test	Result for 2nd isolate (*S. aureus*)
Citrate	+	Glucose	+
Catalase	+	Lactose	+
Urease	+	Mannitol	+
Coagulase	+		

KEY: + = Positive - = Negative

Identification of *Aspergillus niger*:

The initial fungal growth obtained on the damped bread was transferred on a sterile Sabouraud dextrose agar plate. After a span of 3 days, the plate showed the presence of black fungal growth. A few spores when stained with lacto-phenol cotton blue and observed under the microscope. Smooth, round, brown spores, a filamentous structure (hyphae) and presence of dark conidial heads were observed. The isolate was confirmed as *Aspergillus niger.*

Dectection of flavonoids using NaOH:

Presence of flavonoids in each extract was detected using NaCl and HCl. On addition of NaOH, the colour of the extracts turned yellowish-green and on addition of 1-2 drops of HCl, turned the solutions turned colourless, which confirmed the presence of flavonoids.

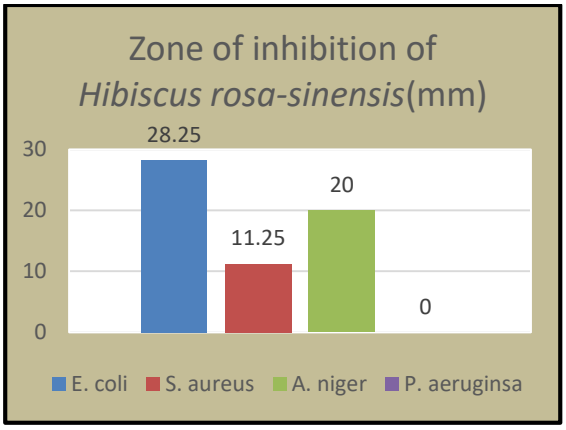
Antimicrobial test:

Antimicrobial activity of all the plant extracts was investigated. Antibiotics like vancomycin, ampicillin and kanamycin were used as controls and were tested against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, respectively, to compare their antimicrobial activity with that of the extracts.

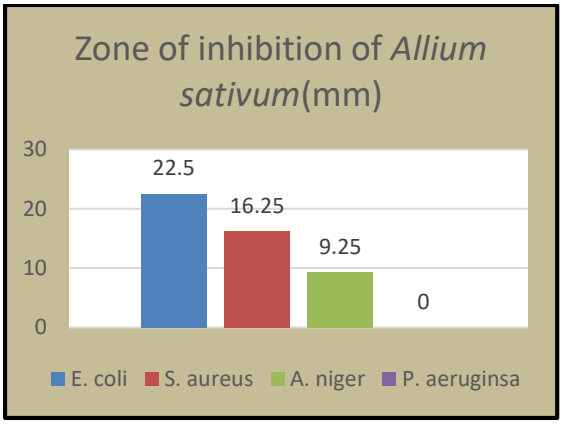
Flavonoids extracted from ginger, garlic, hibiscus and black pepper were tested against *S. aureus, A. niger, P. aeruginosa* and *E. coli* and the following zone of inhibitions (mm) were obtained:

Table 3: Antimicrobial activity of plant extracts against isolates

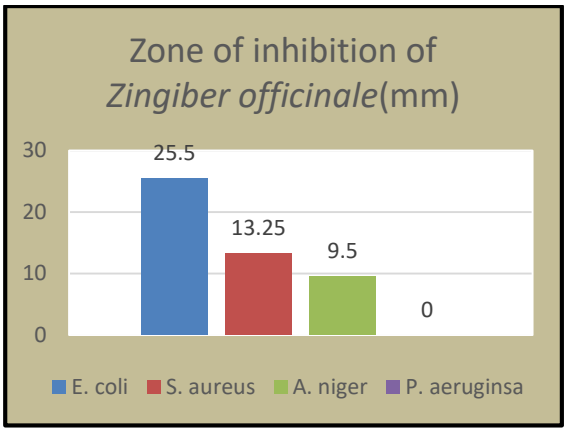
ORGANISM	HIBISCUS	GINGER	GARLIC	PEPPER	ANTIBIOTIC
E. coli	28.25	25.50	22.50	0	34.50
S. aureus	11.25	13.25	16.25	7.50	21.75
A. niger	20.00	9.25	9.25	6.5	_
P. aeruginosa	0	0	0	0	5.5



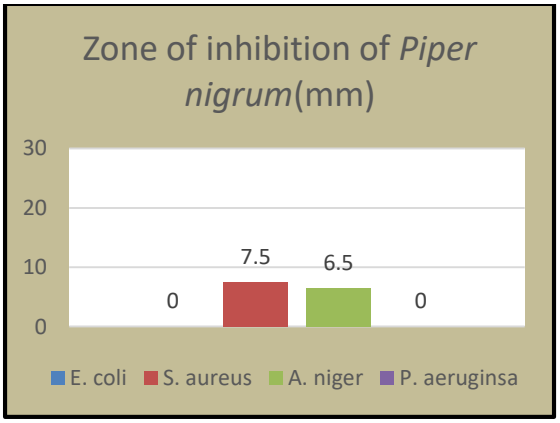
Graph 1: Zone of inhibition of Hibiscus



Graph 3: Zone of inhibition of Garlic



Graph 2: Zone of inhibition of Ginger



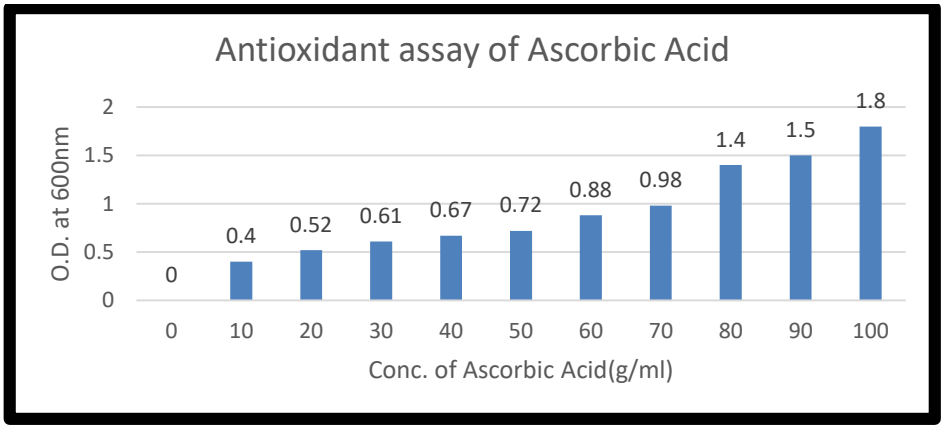
Graph 4: Zone of inhibition of Pepper

Flavonoids present in *Hibiscus rosa-sinensis* provided better antimicrobial activity followed by *Zingiber officinale* and *Allium sativum*. Flavonoids present in *Piper nigrum* were the least effective. Growth of

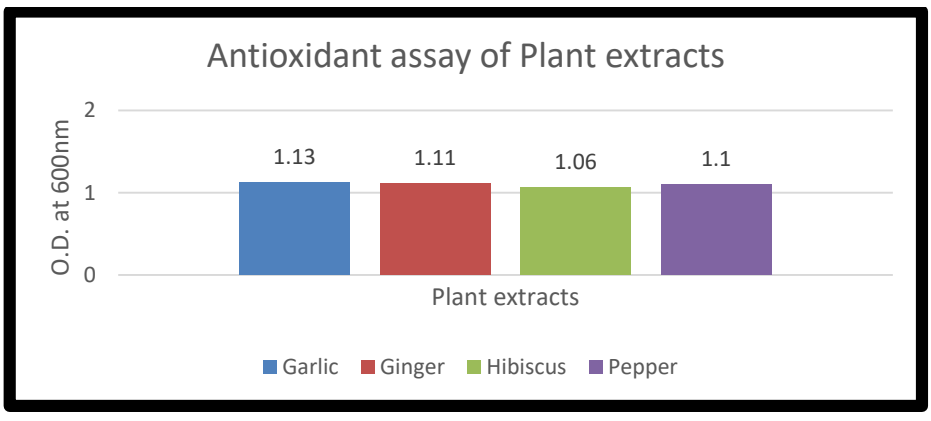
P. aeruginosa was not inhibited by any of the extracted flavonoids.

Determination of antioxidant activity of extracts:

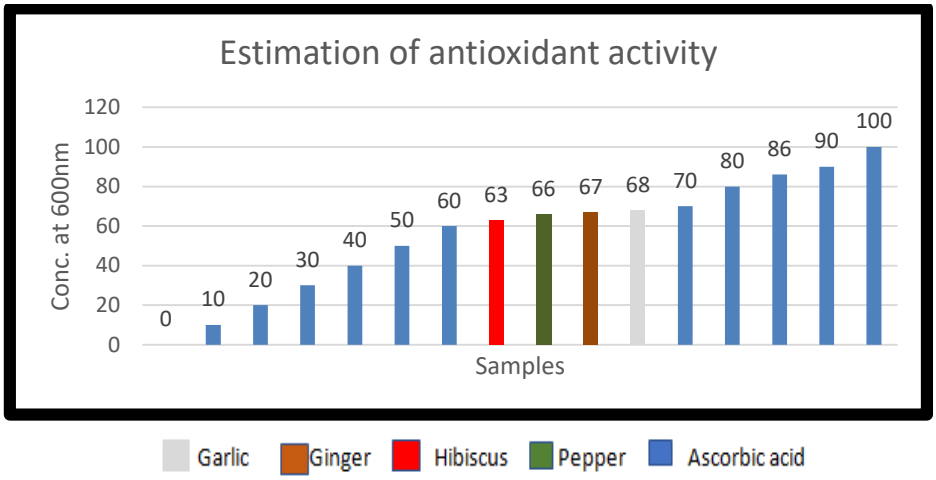
The total antioxidant capacity of the extracts was determined by the phosphomolybdenum assay. Ascorbic acid was used as a standard. On addition of the phosphomolybdenum reagent, an occurrence of blue colour was observed, which gradually increased on heating the test tubes for a period of 90 minutes. Using the standard results and the O.D. of the plant extracts, standard graphs were plotted. The graphs (5), (6) and (7), illustrate the antioxidant activity of the plant extracts as follows: Garlic > Ginger > Pepper > Hibiscus.



Graph 5: Antioxidant assay of Ascorbic Acid

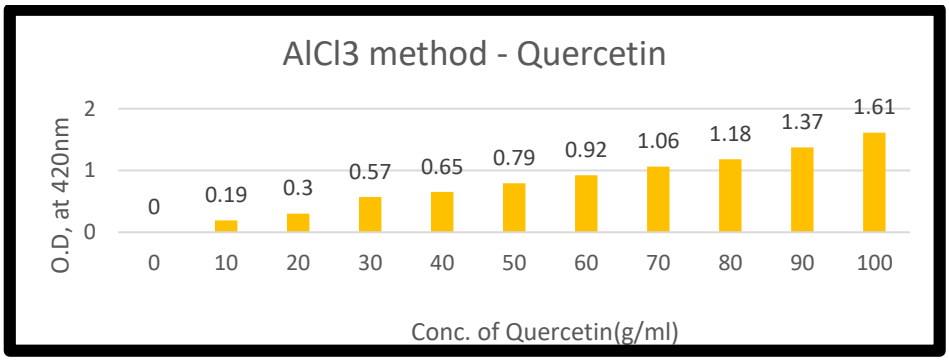


Graph 6: Antioxidant assay - Plant Extracts v/s O.D.

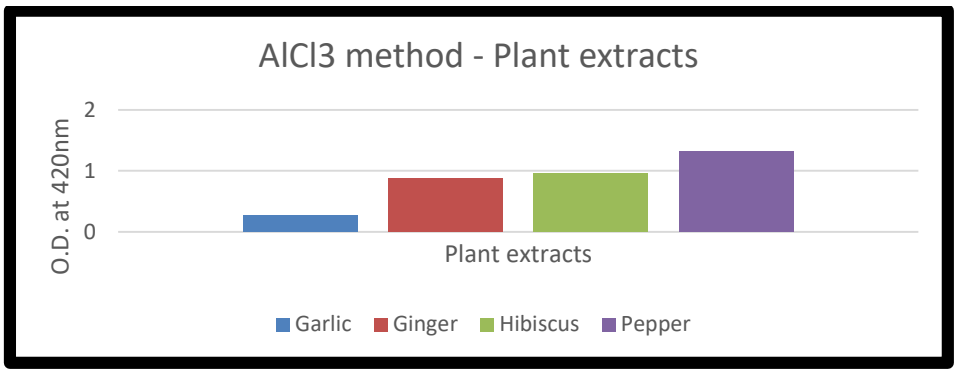


Graph 7: Estimation of Antioxidant Activity using Phosphomolybdenum assay

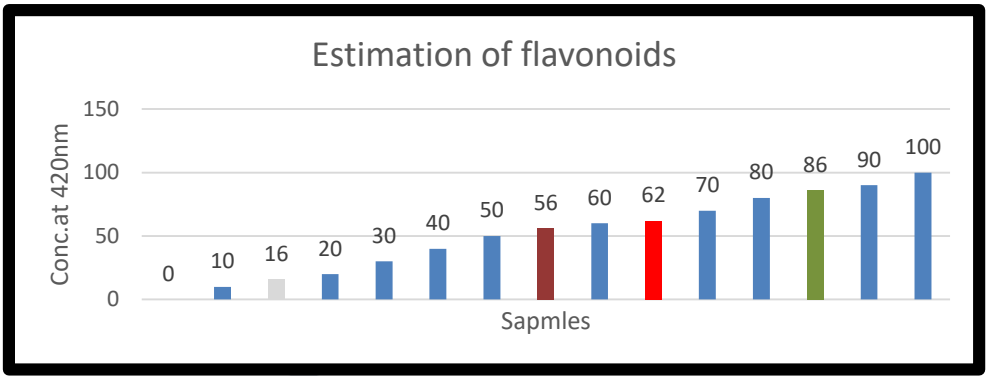
7.Estimation of flavonoids using $AlCl_3$: Flavonoid content of all the plant extracts was estimated by the $AlCl_3$ method. Quercetin dehydrate was maintained as a standard. Using the standard results and the O.D. of the plant extracts, standard graphs were plotted. The graphs (8), (9) and (10), illustrate the flavonoid content in the plant extracts as follows: Pepper > Hibiscus > Ginger > Garlic.



Graph 8: Concentration of Quercetin dihydrate v/s O.D.



Graph9: Flavonoid estimation - Plant extract v/s O.D.



Graph 10: Estimation of flavonoids using $AlCl_3$ method

DISCUSSION

The present study deals with the antimicrobial evaluation of plants (garlic, ginger, black pepper and hibiscus); against *S. aureus*, *E. coli*, *P. aeruginosa* and *A. niger*. Among these, *S. aureus* and *A. niger* were sensitive to all the plant extracts, whereas *P. aeruginosa* was resistant. *E. coli* was sensitive to ginger, garlic and hibiscus but was resistant to pepper. In case of *S. aureus*, the garlic extract produced a zone of inhibition (ZOI) of 16.25mm, which was the largest as compared to other plant extracts. However, in case of *A. niger*, the hibiscus extract produced a ZOI of 20mm. The ginger and the garlic extracts produced almost equal ZOI of 9.25mm each; and the pepper extract produced ZOI of 6.5mm, indicating that *A. niger* was the most sensitive against the hibiscus extract. When considering *E. coli*, almost similar ZOI were observed for all the plant extracts, except pepper, which did not produce a ZOI at all.

The mechanism of antibacterial action of spices and derivatives is not yet clear (Lanciotti, et al., 2004). Hypothesis have been proposed by different workers (Odhav, et al., 2002) involves: hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer; perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes; membrane disruption; destruction of electrons transport systems and cell wall perturbation.

The fungistatic or fungicidal effect of spices is due to the inhibitory action of natural products and the mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes. It is

also reported that plant lytic enzyme act in the fungal cell wall causing breakage of β-1,3 glycan, β-1,6, glycan and chitin polymer (Brull and Coote, 1999).

The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Many plants have been used because of their antimicrobial traits, which is due to compounds synthesized in the secondary metabolism of the plant, such as phenols, essential oils, terpenoids, alkaloids and flavonoids.

In the present study, flavonoids were extracted and their concentration in different plants was estimated using $AlCl_3$ method and then their antimicrobial activity against the selected organisms was tested. Pepper was found to have maximum concentration of flavonoids followed by hibiscus, ginger and garlic. Flavonoid estimation was carried out using a well- known flavonoid, quercetin, as standard. The antimicrobial activity due to flavonoids may be because of their structure, as they have the ability to form a combined complex with bacterial cell walls. Because they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms.

The antioxidant activity was estimated using an antioxidant, ascorbic acid, as standard and was exhibited by all the plant extracts used for the study. Garlic was found to have the highest antioxidant potency followed by ginger, pepper and hibiscus. In last few decades research on spices has been directed to understand their medicinal and antioxidant properties.

Thus spices like ginger, pepper and garlic can protect the human body against cellular oxidation reactions, bacterial infections and other metabolism related disorders.

The present study concludes these extracts could inhibit human pathogens growth such as *S. aureus*, *E. coli* and *A. niger*. The results are encouraging but precise assessment is utterly necessary before being situate in practice as well as the most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo secondary pharmacological evaluation. It is also important to note that the experiment was affected by various factors, which include concentration of spice extracts, volume of agar, and concentration of culture and incubation times. Future studies should be conducted in a

more controlled environment were these factors are constant.

CONCLUSION

The present study concluded that flavonoids can be subjected as a natural substitute to ward off oxidative stress, and micro-organisms involved in skin related diseases.

The presence of flavonoids in all the plant extracts were detected using NaOH and HCl, by observing the colour change. $AlCl_3$ method was used to estimate the flavonoid content in each extract and was found to be in the following order: Pepper > Hibiscus > Ginger > Garlic, which shows that pepper has maximum flavonoid content whereas garlic has the least. The antioxidant activity of flavonoids is accredited to their redox properties. This activity was determined by the phosphomolybdenum assay. It was observed in the following order: Garlic > Ginger > Pepper > Hibiscus, wherein the antioxidant activity of all the plant extracts was more or less the same, with garlic being the highest and that of hibiscus being the least.

The results obtained from antimicrobial test, using the agar cup method showed different micro-organisms exhibiting different sensitivities towards flavonoids. Flavonoids extracted from all the plant extracts proved to be effective against *S. aureus* and *A. niger*, whereas except pepper, flavonoids from the rest of the plant extracts were effective against *E. coli*. But none of them proved to be effective against *P. aeruginosa*. Flavonoids present in *Hibiscus rosa-sinensis* provided better antimicrobial activity, followed by *Zingiber officinale* and *Allium sativum*, whereas flavonoids present in *Piper nigrum* were the least effective against the tested micro-organisms. Different types of flavonoids are present in different plants in varying quantities, which imparts contrasting levels of antimicrobial and antioxidant activities. Hence, though the flavonoid count is less in some plant extracts, they still exhibit strong antioxidant and antimicrobial activity.

Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent antimicrobial agents. Thus, spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like microbial pathogens. These extracts must be investigated

further to check in vivo efficacy and safety before it is used for commercialization.

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