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Screening for Phytochemical, Anti-oxidant and Anti-microbial Activity of Zingiberaceae Sample Extracts

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Abstract: ***Diethyl Ether and Methanol extracts of Zingiberaceae species from the Curcuma Longa (Tumeric), Zingiber Officinale (Ginger) and Nelumbo Nucifera (Lotus) were partitioned for anti-microbial and anti-oxidant activities. Turmeric, Ginger and Lotus roots which are of rich rhizome content were used for relative analysis of antioxidant and antimicrobial activity. Anti-oxidant activities was firmed by DPPH assay. The result shows rhizomes are more effective in contradiction of Escherichia Coli Pseudomonas Aeuroginosa, Bacillus Subtilis, Salmonella Typhi and Staphylococcus Aureus paralleled to other plant extracts. Preclinical readings of Zingiberaceae extractions shown analgesic properties that purposes to systematically evaluate and meta-analyze whether extracts from Zingiberaceae are clinically effective hypoalgesic agents. Zingiber Officinale is a communal condiment in various nutrients and liquid refreshments. It is used in traditional Medicine herb for the treatment of stomach disorders. The ingredients present in ginger, turmeric and lotus have high potent anti-oxidant and anti-inflammatory activities that are used in cookery. Thus, the study deals with antimicrobial activity of all the three sample extracts and their phytochemical composition.***

Keywords: Antimicrobials, Phytochemical Properties, Staphylococcus Aureus, Streptococcus, Zingiber Officinale, Nelumbo Nucifera and Curcuma Longa, Antioxidant Assay.

I. INTRODUCTION

An introduction to determine the extraction of rhizome contents in three different types of roots includes Ginger (*Zingiber officinale*), Lotus (*Nelumbo nucifera*), Turmeric (*Curcuma longa*). It is grown in shoots and the roots of plants stem underground and these stem-tubers were the adapted system of rhizomes used in the storing purposes by plants.

Gingers of the family Zingiberaceae are perennial herbs that produce aromatic rhizomes. Rhizomes in plants can be consumed in raw or can be cooked as vegetables, even used for condiment foods. Gingers of the family Zingiberaceae are perennial herbs that produce aromatic rhizomes. Ginger plants are widely used as spices, condiments, and traditional medicines. The medicinal uses of rhizomes and leaves of gingers have been reviewed. Rhizomes of Z. Officinale are used as seasonings and condiments in the nutritional and liquid refreshment industries. They are used in the production of beverages such as ginger beer, ginger algae, and ginger wine.

Lotus is an anti-oxidant which determines the dietary fiber content in cookery sausages and its very efficient natural component in meat foodstuffs for the progress of good health and useful foods. It is a sacred plant which has a high medicinal qualities and is determined by its elongated floating leaves with a bright colored fragrance in flowers. Its flowers are grown on top with its leaves floating on water with very long stems with air spaces in it and its functionalities are used in control of bleeding and, even in digestive problems.

Turmeric is a bio-active which represents a polyphenol-curcumin, extracted with the use of organic solvents. It has a anti-inflammatory assets, it is rarely bio-available can be used as mediated through secondary metabolites. It can be used in a traditional style of medicines which helps to determine the efficiency of ginger extracts on particular human pathogens, such as *Staphylococcus aureus* and *Streptococcus* species.

II. MATERIALS AND METHODS

A. Collection of Plant Materials

The materials are wrapped in a plastic bag, kept in the refrigerator and brought to the laboratory for analysis of next day. Leaves and rhizomes (100 g each) were washed thoroughly with distilled water and ragged into 0.2 cm shreds using a pasta manufacturer.

B. Preparation of Turmeric, Ginger and Lotus Root Extract

The herbal plants including Ginger, Turmeric, and lotus roots were washed with distilled water and were allowed to dry in absence of daylight at 30-350° Celcius. The plant extract is peeled and cut into small pieces by using mortor and pestle, the extract is transferred into conical flask and 300 ml of methanol extraction is added. Magnetic stirrer is added to it and covered using aluminium foil.

C. Extraction Method

The roots of ginger used for this study were washed with distilled water, dried in shade and then ground to powder. About 200g of powder was distinctly soaked in 400 ml of 95 % methanol, along with distilled water and diethyl ether in a 500 ml of reagent bottle and then it is stoppered. It was allowed to stand still for 14 days to permit complete abstraction of the active materials. The sample for cold extract was kept for 12 hours and then these extract were stores in the refrigerator the whole night. After that, the extraction was taken and filtered using filter paper. The sample extraction was measured by measuring cylinder.

D. Distillation Method

The extracted plant solution is taken and the solution is transferred into a round bottom flask. The extracted solution should get reduced to 250 ml from round bottom flask. The liquid methanol gets condensed into vapour form and clears the solution in the beaker. After the sample gets cooled shake the flask is shook clearly, and then transferred it into a vial and the solution stored in the refrigerator.

E. Antimicrobial Assay

The antimicrobial properties of the extracts were determined using the agar diffusion and the diffusion disc methods. In agar diffusion method, there will be a 24 hour old broth-cultures of the given organisms were swabbed on a sterilized Mueller-Hinton agar in petri-plates by using sterilized cotton cloth. A sterile cork awl of 6 mm diameter was used to punch wells on the agar on each of the petri dishes. The holes were filled with 80 ml of each sample extracts.

Diffusion discs are then impregnated with the same concentrations of extracts with the agar diffusion method which is 0.6µg/ml, 0.4µg/ml and 0.2 µg/ml and also with 80ml of each sample extract which is used as the control. Dics are uniformly distributed and informally pressed on agar surfaces. The procedure were passed for each extract and, inoculated petri-dishes were leftout for few more minutes for extraction to the diffusion into an agar. The petri-plates are nurtured at 37° Celsius for twenty-four hours, after which the zones of the process in inhibition were dignified.

F. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined against bacteria after the antimicrobial test was performed. MIC is the lowermost absorption of an anti-microbial that inhibits the noticeable growth of micro-organisms after over-night period of incubation. MBC is the lowermost concentration of an anti-bacterial agent which is essential to slay a specific bacterium.

For MIC determination, Mueller Hinton agar was used and agar diffusion method was used for the test. Sterile Cork tool of a diameter of 6 mm were used to drag holes on the petri-plates after planting the plates with bacterial-strains concerned, which are leftout for about an hour at a room temperature and incubated at 37 °C. The results can be seen after 24 hours.

III. PHYTOCHEMICAL SCREENING

The plant extractions that are collected were processed to phyto-chemical analysis of various active components to carry out a standard identification of various microbial species.

A. Determination of Alkaloids

Alkaloids are determined with a 2 ml of methanol extract and a few droplets of wagners reagent that were added in it. It forms a reddish-brown precipitate which specifies the presence of alkaloids in it.

B. Determination of Flavonoids

An intense form of yellow color is observed with the mixture of a 2 ml of each type of extract with a few droplets of 2% NaOH added. A few droplets of dilute sulphuric acid (HCl) with 70 % was added which makes the yellow color to get disappeared. The disappearance and its formation of yellow colour in extract tests indicates the incidence of flavonoids in extract.

C. Test for Saponins

Bubble formation with the foam with persistency was formed by adding a 2ml of the extract of each type with 6ml of the distilled water that was added vigorously which specifies the occurrence of saponins.

D. Test for Tanins

Creation of brownish blue or a black in color by adding 1ml of each type of extract with 10% of the alchoholic ferric chloride which was added in it, that specifies the occurrence of tanins.

E. Test for Phenols

Formation of a blue colored solvent by adding 1ml of each form of extract with 1ml of the 5% of aqueous ferric chloride that was added in the mixture which specifies the occurrence of phenols.

F. Test for Proteins

Formation of a violet colored precipitate by adding a droplets of 2ml of each form of extract with 1ml of the 40% of caustic soda (NaOH) and a few droplets of 1% of (copper sulphate) $CuSO_4$ added in it determines the occurrence of proteins.

G. Test for Quinones

Formation of a yellow color precipitate or an extra coloration by adding a 1ml of each type of extract and a few droplets of a concentrated H_2SO_4 that was added in to it creates the essence of quinones.

H. Test for cardiac glycosides

Formation of a brown colored ring at the interface which indicated the occurrence of a deoxysugar that is the characteristic of cardenolides by adding a 0.25 grams of each form of extract that is diluted to 5ml of water with 2ml of the glacial acetic acid that containing 1 droplet of ferric chloride solvent added which was under lined with 1ml of H_2SO_4. A violet kind of ring that seemed below the brown kind of ring formed, while in the acetic acid layer, a greenish kind of ring may form that is just above the brown ring, and that gradually spreads throughout this layer.

IV. QUANTITATIVE PHYTOCHEMICAL ANALYSIS

A. Total Alkaloid Content Determination

A mixture of 40 ml by its 10% of acetic acid with ethanol were added with 1 gram of powdered form of samples that are covered to stand for about 4 hours allowance, and then these filtrates were concentrated in water bath for $1/4^{th}$ of the original volume.

B. Total Flavonoids Content Estimation

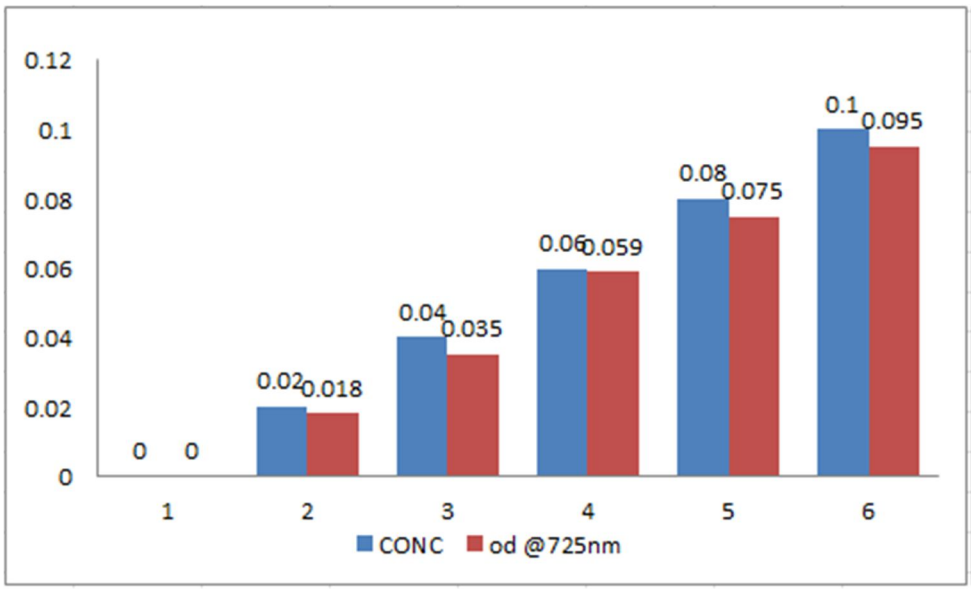
A mixture of 5 ml of 2 % AlCl3 prepared in methanol was mixed with same volume of extract solution. After 10 min, absorbance was measured at 415 nm against blank. Blank was prepared as 5 ml extract mixed with 5 ml of methanol with $AlCl_3$. Catechin was used to plot the standard graph**.**

C. Total Carbohydrate Determination

For process of the polysaccharide content, a mixture of 1 ml of 5% phenol to 1 ml of sample solution was added then, 5 ml of H_2SO_4 added to it and then measure the absorbance of it after 10 mins at 488 nm alongside the blank. After which compare it with a standarded solution of glucose. And to prepare the Blank, add 1 ml of distilled water to 1 ml of the 5 % phenol along with 5 ml of H_2SO_4.

Table 1: CH2O Determination table

Sl.no	Concentration(mg/ml)	Absorbance OD@488nm
1	2	0.018
2	4	0.035
3	6	0.059
4	8	0.075
5	10	0.095
6	12	0.090



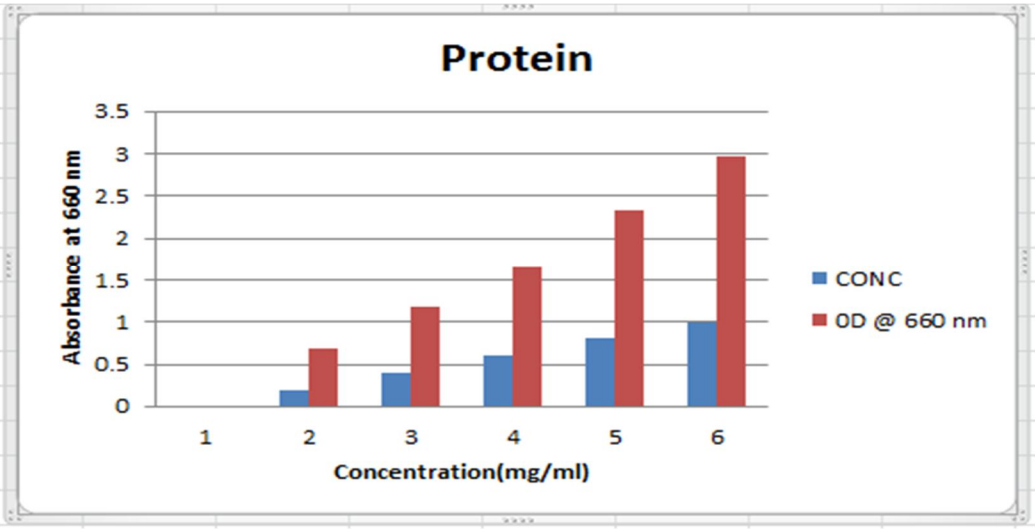
Graph 1: CH2O Determination

D. Total Protein Content Determination

The total protein content was determined by Bradford's method. Briefly, to the 100 µl of the sample, extract add 3 ml of bradford's reagent and incubate in dark for 5 minutes. The absorbance value was measured at 595 nm. Bovine serum albumin was used as the standard solution.

Table 2: Protein Content Determination

Sl.no	Concentration(mg/ml)	Absorbance OD@595nm
1	0	0
2	0.2	0.695
3	0.4	1.187
4	0.6	1.659
5	0.8	2.33
6	0.10	2.973
7	0.12	2.975



Graph 2: Protein Estimation

V. DPPH ASSAY

A. Principle

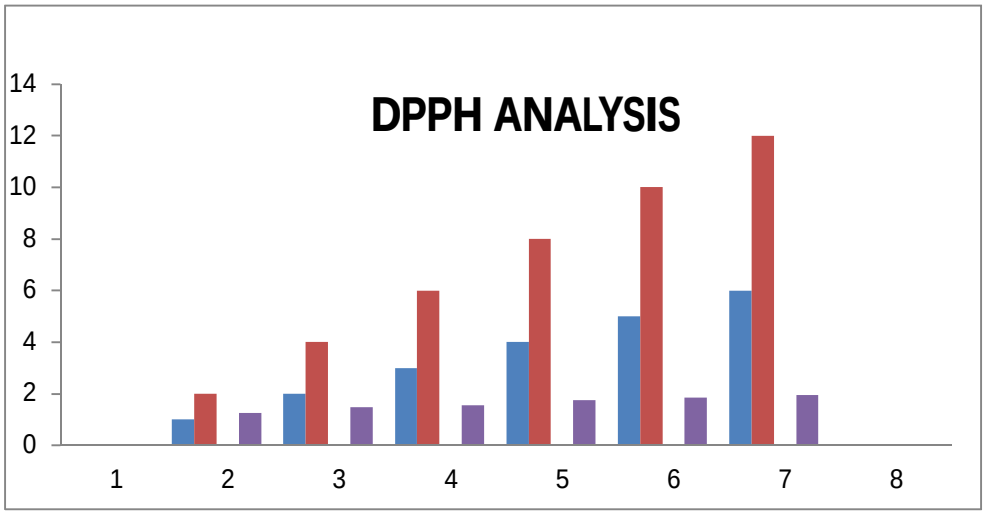
DPPH is characterized as a free radical which has maximum absorbance at 517nm. When the antioxidant compound reacts with the free radical, it will donate one proton to the free radical and hence the free radical will get scavenged. So the color changes from violet to pale yellow.

B. Procedure

Method was based on reduction of stable DPPH radical antioxidants in a methanol solution. In the presence of antioxidants, the reduction of the DPPH radical solution was monitored. 1 ml of 0.1 Mm DPPH solutions was taken and 1 ml of different concentrations (100 – 1000 µg/ml) of sample was added. The reaction mixture was incubated in the dark for 30 minutes. The change of colour from purple to yellow was read at A 517 nm.

Table 3: DPPH Assay

Sl.no	Concentration(mg/ml)	Absorbance OD@517nm
1	2	1.25
2	4	1.47
3	8	1.56
4	10	1.76
5	12	1.89
6	14	1.95



Graph 3: DPPH Assay

C. Antioxidant Activity

10 mg/ml of Ascorbic acid was used as a reference to compare results of the plant extracts. In 3 ml of total reaction solution, 2 ml of extract/standard solution and 1.0 ml of DPPH were mixed and allowed to react at 37 °C for 30 min. Later the absorbance value was measured at 520 nm and converted into percent antioxidant activity. The percentage anti-oxidant activities was calculated by the following formula: Percent (%) inhibition of DPPH activity = (A – B)/A *100 Where A = Absorbance of the blank and B = Absorbance of the sample.

D. Agar Well Diffusion Method

It is used with 0.5 ml of 48 hours old culture test organisms were inoculated into different sterile petri-plates and about 20 ml sterile media was poured into each dish. The dishes were gently shaken for proper mixing and allowed to solidify. Thereafter, four wells of 5 mm diameter were punched mm with a sterilized cork borer. For each well, 50 µl of different extracts were added. The plates were incubated at 37 °C for 24 hours and then zone of inhibition in mm was measured. It is defined in triplicates.

E. Spectrophotometric Method

80 ml strains of 48 hrs old cultures were inoculated in test tubes with 20 ml culture media. To each tube, 40 µl of different extracts were added and incubated for 24 hrs. Optical density of grown bacteria was measured at 450 nm wavelength.

VI. RESULTS AND DISCUSSION

A. Results

The comparative study of antimicrobial and antioxidant activity was conducted with dietary plants like lotus, Ginger, Turmeric. We have also evaluated the antimicrobial activity by diffusion and spectrophotometric method. All the experiment were done in triplicate.

B. Antioxidant Activity

Among the five extract of plants, ginger showed maximum antioxidant activity showed the lowest activity and reported as 51.54 % for turmeric, 28.17 % for lotus, 54.08 % for ginger, 10.97 %. Further experiments are needed to confirm which phytochemicals to show antioxidant activity. There are major articles available that report that these plant extracts have antioxidant activity.

C. Antimicrobial Activity

Agar well diffusion method shows the antimicrobial activity of selected plant extracts by agar cup diffusion method and the result is similar to that of spectrophotometric method. The results reveal that Garlic extract inhibit maximum growth of E. coli whereas Pseudomonas aeruginosa is inhibited maximum by Aloe and Flower bud extract which inhibit maximum growth of Bacillus subtilis whereas Staphylococcus aureus is maximum inhibited by Ginger. The antimicrobial activity of methanolic extract of turmeric, lotus, ginger, were tested against bacterial strain such as Escherichia coli, Pseudomonas aeuroginosa, Bacillus B. subtilis and Staphylococcus auerus by a spectrophotomter.

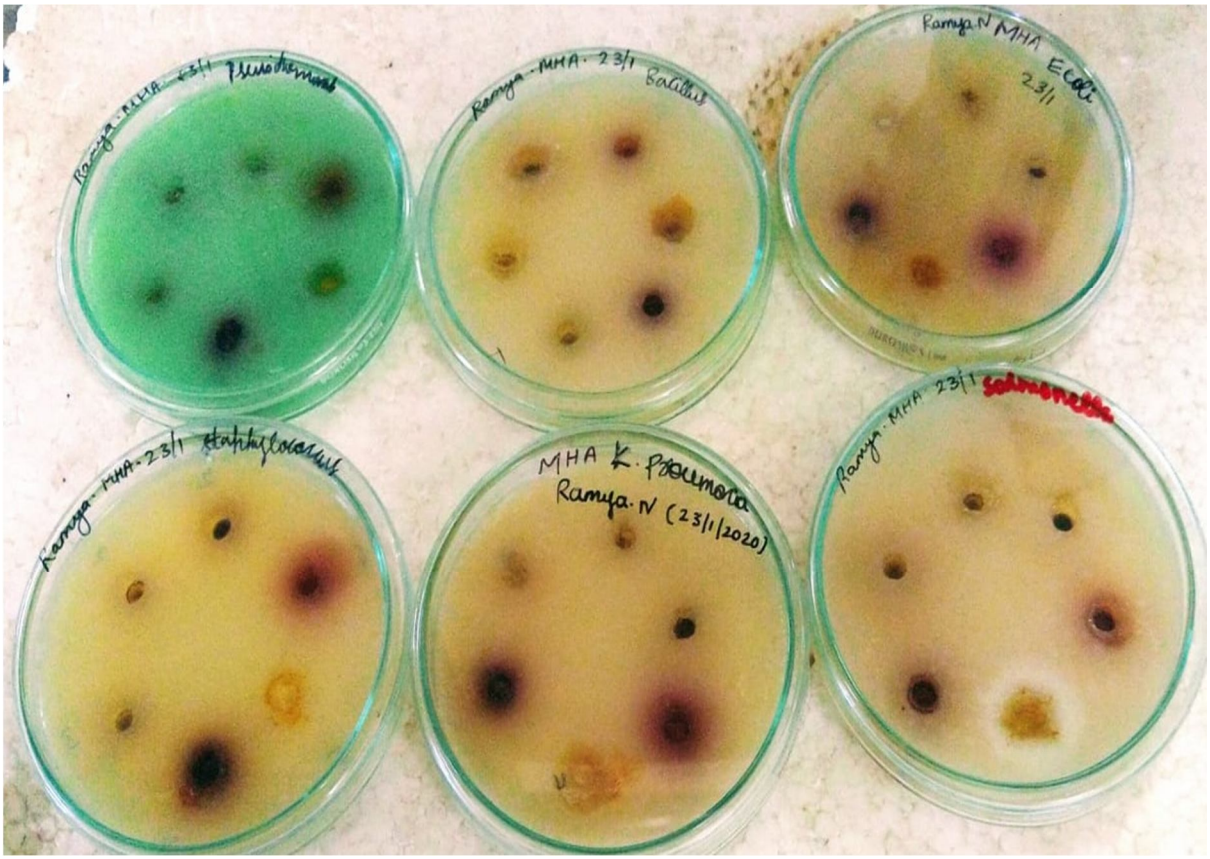


Figure1: Antimicrobial activity by using agar diffusion method

Table 4: Preliminary phytochemical screening of Zingiberaceae samples from different solvent

Phytochemical constituents	Different solvent extracts	
Alkaloids	+	+
Terponids	+	-
Glycosides	+	+
Saponins	+	-
Flavonoids	+	+
Tanins	+	-
Phenols	+	+
Proteins	+	+
Carbohydrates	+	+
Glycin	+	+
Alchol	+	+

Antimicrobial efficacy of the various solvent extracts namely ethanol, ethyl acetate and acetone, has been investigated against few human pathogenic microbes. It is evident that ethanol extract showed maximum zones of inhibition ranges between 7.83±0.47mm and 17.83±1.04mm.

Table 5: Antimicrobial activity of leaf extracts of Zingiberaceae

Microbial strains	Zone of inhibition (mm)	
	Methanol	Diethyl ether
Bacillus subtilis	16.00±1.00	8.83±0.76
Staphylococcus aureus	17.83±1.04	10.50±0.50
Escherichia coli	8.00±0.00	10.50±0.50
Pseudomonas aeruginosa	8.16±0.76	17.00±1.00
Klebsiella pneumoniae	7.83±0.47	10.50±0.50
Streptococcus faecalis	8.33±1.04	8.50±0.50

D. Discussion

The phytochemical screening result revealed that ethanolic extracts of plants consist phenols, flavonoids, Saponins, tannins, glycosides, terpenoids, alkaloids and carbohydrates. In the antimicrobial result, it was observed that all ethanol extracts of plants demonstrated good antibacterial activity. The result of antimicrobial activity expressed significant result against bacterial strains. From the determination of antimicrobial activity it was observed that growth of E. coli and S. auerus is inhibited most by garlic whereas P. aeuroginosa inhibit growth of B. Subtilis the most. Turmeric and ginger also showed significant antimicrobial activity. In comparing the well diffusion and spectrophotometric method, well diffusion method has ability to evaluate the activity of antimicrobial drugs including plant extracts. This method has no significant difference from other methods according to some reports. Spectrophotometric method of finding the inhibitory action of drugs and extracts is fast and comfortable to use. Statistically, there is no significant difference between results obtained by both methods. So, spectrophotometric method can be recommended as a suitable and sensitive method for investigation of antimicrobial activity of extracts. The scavenging of DPPH radical is most used protocol to evaluate free radical scavenging ability of plant extracts. The result of antimicrobial activity expressed significant results against bacterial strains. From determination of antimicrobial activity, it was observed that growth of E. coli and S. auerus is inhibited most by ginger whereas P. aeuroginosa inhibit growth of B. Subtilis the most. Turmeric and ginger also showed significant antimicrobial activity. The phytochemical screening results revealed that ethanolic extracts of plants consist phenols, flavonoids, Saponins, tannins, glycosides, terpenoids, alkaloids and carbohydrates.

VII. CONCLUSION

In conclusion, the work revealed that methanolic extracts of plants are dissimilar in their antimicrobial and antioxidant properties. The study revealed that turmeric and ginger have higher antioxidant activity but there is variation in antimicrobial activity in specific organism. However, further studies are necessary in vitro and in vivo to support this suggestion. Further, phytochemicals analysis should be done to qualify and quantify the component which possesses antimicrobial and antioxidant activity at molecular level. This result show that these plants are as good source of antimicrobial and antioxidant agents and spectrophotometric technique is effective for determination of antimicrobial activity of extracts.

Our findings indicated that *Zingiberaceae* extracts are clinically effective hypoalgesic agents and the available data shows a better safety profile than non-steroidal anti-inflammatory drugs.

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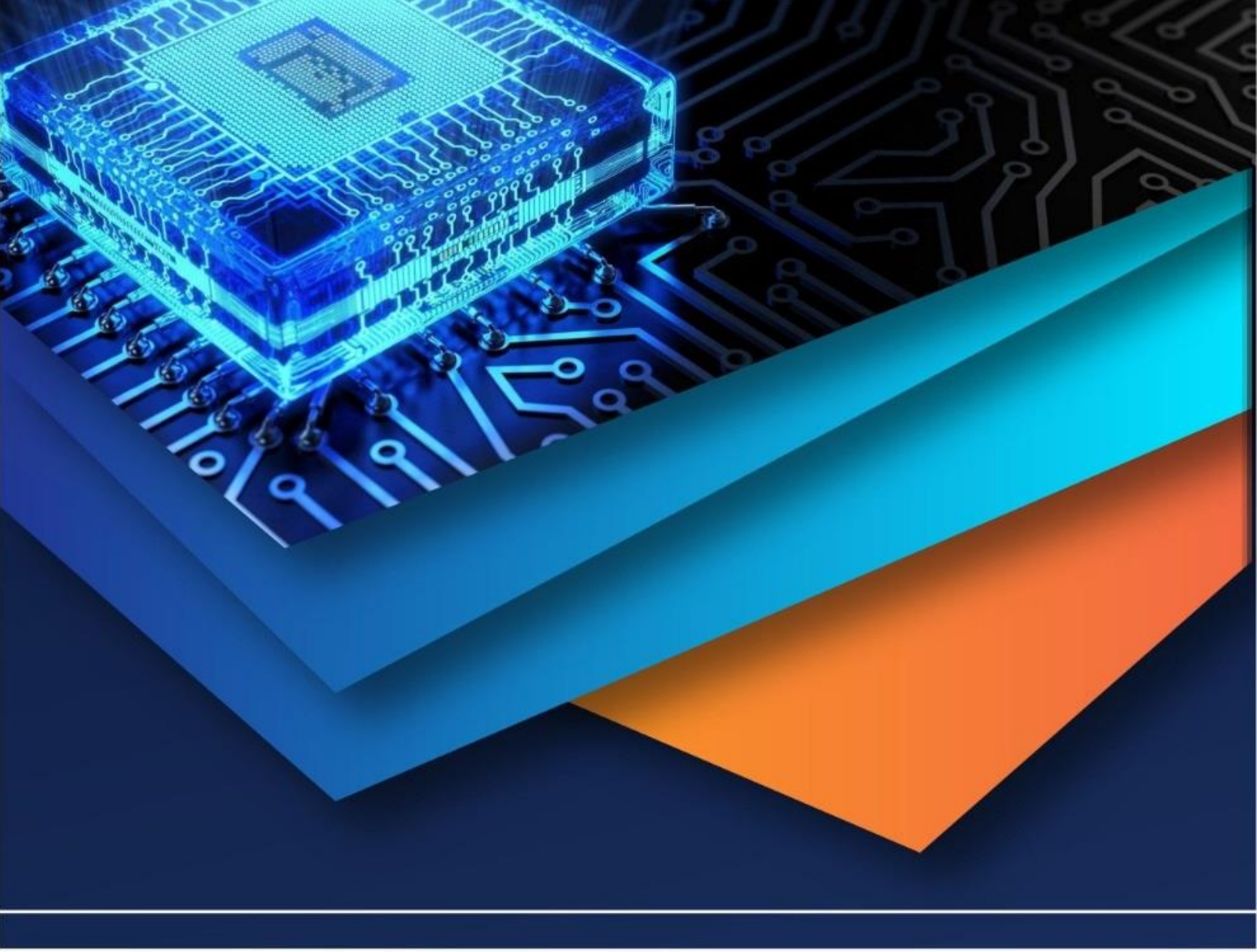

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