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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY PROFILE OF CHICKEN FERMENTED WITH *Syzygium aromaticum.*

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Abstract – Clove (Syzygium aromaticum (L.) Merril. & Perry, syn. Eugenia aromaticum or E. caryophyllata) is one of the most ancient and valuable spices of the Orient.Clove (Syzygium aromaticum, L.) is known for its antimicrobial activity against several pathogenic bacteria, has been known for its antioxidant property. The aim of this study was to assess the antioxidant and antimicrobial properties of after fermentation of minced chicken meat with Syzygium aromaticum. Results indicated that chicken prepared after fermentation of minced chicken meat (without spices) fermented with Syzygium aromaticum showed higher antioxidant scavenging activity against hydroxyl, and DPPH free radicals activity of Syzygium aromaticum. Minced chicken meat (without spices) fermented with Syzygium aromaticum showed the bactericidal effect against Enterococcus, E.coli, Klebsella pneumonia.The results indicated that enhancing the antioxidant and antimicrobial potential of Syzygium aromaticum in minced chicken meat during fermentation.

Keywords: E. caryophyllata, Syzygium aromaticum, antimicrobial activity,antioxidant activity, Minced Chicken meat.

1. INTRODUCTION

Syzygium aromaticum is commonly known as clove, belong to a family of Myrtaceae. Cloves are aromatic flower buds of tree. They are native to the Maluku islands in Indonesia and are commonly used as spice. The clove tree is an evergreen that grown upto 8-12 meters (26-40 ft) tall, with large leaves. Clove has been used as primary preservatives, are high in antimicrobial, antifungal and antioxidant activity etc. This plant is also used as medicinal herb has it contain many health benefits such as improve digestion, fights lung cancer, and for treating oral diseases etc., Fermentation is a food processing technology that utilizes the growth and metabolic activity of microorganisms for the stabilization and transformation of food materials. Fermentation was primarily developed for the stabilization of perishable agricultural produce. Notwithstanding, the technology has evolved beyond food preservation into a tool for creating desirable organoleptic, nutritional, and functional attributes in food products. Fermented food products still make up a significant portion of the diet in developing countries and the Far East, whereas that is no longer the case in the developed West. Nevertheless, there is a renewed interest in fermented food products in recent times mainly driven by the purported health benefits of such products. The current trend is set to continue into the future in light of the increasing prevalence of metabolic syndromes such as obesity, various food allergies, and intolerances (lactose intolerance, gluten intolerance, etc.) life style choices such as vegetarianism and veganism; and increasing interest by consumers in everything perceived natural and that promotes health and longevity. Fermentation is a metabolic process in which an organism converts a carbohydrate, such as starch or a sugar, into an alcohol or an acid. For example, yeast performs fermentation to obtain energy by converting sugar into alcohol. Bacteria perform fermentation, converting carbohydrates into lactic acid.

2. Materials and methods:

PLANT SAMPLE COLLECTION AND PROCESSING:

The Clove Syzygium aromaticum were collected from local market in Redhills, Chennai. The collected clove was treated and unwanted dust particles were removed. The cleaned clove were shade dried for a period of 15 days, it was shade dried in order to prevent the denaturation of phytochemicals due to heat. After a period of 15 days, the clove bud were homogenized into powder by using mortar and pestle. The mortar and pestle were chosen to grind the clove bud because the heat generation, during grinding is lower compared to other grinding procedure. The obtained dry powder was about 45g and it was stored for further usage.

PLANT EXTRACT PREPARATION:

The plant extract was prepared by using a method called Soxhlet extraction. The solvent chosen for the procedure of Soxhlet extraction in order to extract all the phytochemicals from the clove buds is water. Water is a strong solvent which has the ability to extract maximum phytochemicals from the sample.

PHYTOCHEMICAL ANALYSIS OF EXTRACT:

Phytochemical analysis is screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

(A)PHENOLIC COMPOUNDS:

(i) Gelatin Test: The extract is dissolved in 5ml of distilled
water and 2ml of 1% gelatin solution containing 10% of NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

(ii) Ferric Chloride Test: The extract is dissolved in 5ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates presence of phenolic compounds.

(B)ALKALOIDS:

(i)Dangendroff's Test: 2ml od Dangendroff's reagent was added to the extract and orange colour indicates presence of alkaloids.

(ii) Mayer's Test: 2ml of Mayer's reagent was added to the extract creamy precipitate indicates presence of alkaloids.

(C) SAPONINS:

The crude extract was diluted with 20ml of distilled water and it was agitated for 15 mins. The formation of 1cm form layer showed the presence of saponins.

(D) FLAVONOIDS:

Few drops of aluminium solution added in the sample. Yellow colour indicates presence of flavonoids.

(E)GLYCOSIDES:

2ml of chloroform was added to extract and concentrated sulfuric acid was added. Reddish brown colour indicates the presence of glycosides.

(F)TANNINS TEST:

2ml of extract was added to 4ml of water and few drops of 0.1% ferric chloride added and forms dark blue colour solution.

(G)TERPENOIDS:

5ml of extract was taken and 2ml of chloroform added and 3ml of concentrated sulphuric acid was added then formation of layer indicates the presence of terpenoids.

3.4 TOTAL PHENOL CONTENT DETERMINATION:

Total phenolic content (TPC) was determined by folin-ciocalteu method.Briefly, an aliquot of the sample extract(0.1ml) was mixed with distilled water (3ml) and 0.5ml of folin-ciocalteu reagent was added after 3min,2ml of 20% sodium carbonate was added and mixed thoroughly.the tubes were incubated in a boiling water bath for exactly 1min, then cooled and the absorbance was measured at 650nm using spectrophotometer (Shimadzu UV 1640) against the reagent blank. Total phenol content was expressed as mg gallic acid equivalent (mg GAE)/100 g sample dry weight.

5 EVALUATION OF ANTI MICROBIAL ACTIVITY:

The antimicrobial activity of clove extract can be analyzed by a method called Agar well diffusion assay which is a common antimicrobial assay to find out the maximum inhibitory concentration of the sample. The antimicrobial activity was analysed against the Staphylococcus aureus, Enterococcus , Klebsella pneumonia, E.coli , a common bacterial organism.

3.5.1 AGAR WELL DIFFUSION ASSAY:

The Agar well diffusion assay is in which the antibacterial activity of the clove extract can be confirmed. In this assay four Muller Hinton agar plates were prepared, initially the glasswares and the media were sterilized using autoclave and the sterilized media was poured on the plates.

After solidification one of the plate was swabbed with Staphylococcus aureus. Similarly all the plates were swabbed with different culture such as Enterococcus, E.coli, Klebsella pneumonia respectively. Four wells were created using well puncher one was maintained as control and at three wells sample was loaded at different concentrations 25ul, 50ul, 75ul respectively.

All plates were incubated at room temperature for 24 hours and the result was viewed and analyzed, thus by measuring the inhibition range the antibacterial efficiency was confirmed.

EVALUATION OF ANTI OXIDANT ACTIVITY:

The anti-oxidant activity of the crude extract of Syzygium aromaticum flower was evaluated

spectrophotometrically using the method of DPPH assay and Hydrogen Peroxide assay.

DPPH ASSAY:

In DPPH(2, 2-diphenyl -1- picrylhydarzyl) assay, the free radical scavenging of the crude extract of S.aromaticum was measured in terms of hydrogen donating or radical - scavenging ability using the stable radical DPPH (Blois, 1958) solution of DPPH in ethanol(0.1mM)was prepared 1.0ml this solution was added to 2.0ml of S.aromaticum flower extract at different concentration (100-500 /ml). After 30 minutes the absorbance was measured at 517nm. Ascorbic acid was used as the positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

HYDROGEN PEROXIDE ASSAY:

The hydrogen peroxide scavenging was determined according to the method of Ruch et al. (1989). A solution of H_2O_2 was prepared in phosphate buffer and their concentration was determined spectrophotometrically from the absorption at 230nm.various concentration of S.aromaticum extract were added to H2O2 and incubated for 10min. The absorbance at 230nm was determined against a blank containing phosphate buffer without H2O2. The percentage scavenging of H2O2 and standard ascorbic acid was calculated using the formula :

Inhibition (%) = [1-(Abs sample /Abs control)]x100

Where Abs sample is absorbance of the sample extract, Abs control is absorbance of the control (ascorbic acid). The sample concentration provides 50% inhibition (IC 50) was calculated by plotting Inhibition percentage against various concentration of the extract used.

TREATING CHICKEN WITH EXTRACT FOR FERMENTATION:

The chicken was collected in local market, Redhills, Chennai. And kept in ice packed container and transferred to lab within 30mins. The chicken was cutted into 10 pieces of approximately 1gm in weight. The small chicken pieces were dipped in the clove extract and wrapped in sterile aluminium foil. And 5 pieces of undipped chicken was wrapped in sterile aluminium foil. The control group consisted of small chicken without any treatment. and stored under refrigeration at 4±1oC.

EVALUATION OF MICROBIAL LOAD IN FERMENTED CHICKEN:

These portions were taken for microbiological analyses on 0, 2, 4, 6 and 8 days of study. At each day of analyses, 1 g sample was homogenized with 225 ml sterile saline water (0.1 percent) to form one in 10 dilution of the sample. Further 10 fold serial dilutions were prepared by transferring one milliliter to nine milliliter of the diluent. Dilutions were made up to 10-5 and selected dilutions of each sample were used for the estimation of various microbial loads per milliliter of sample.

Then the plates were prepared with LB agar, initially the glasswares and the media were sterilized using autoclaved and allowed to cool for 10 mins then 0.1 ml of diluted treated sample was inoculated and the media was poured on the plates. The plates were allowed to solidified and plates were incubated at 37°C based on number of colonies growth the microbial load was determined.

3. RESULTS:

PLANT SAMPLE COLLECTION AND PROCESSING:

The sample of Syzygium aromaticum were collected and shade dried for a period of 15 days. The shade dried leaves were homogenized into powder by using mortar and pestle. The powdered leaves were stored for futher usage.

PLANT EXTRACT PREPARATION:

Plant extract was prepared by Soxhlet extraction method in which the sample was loaded in Soxhlet apparatus and 150ml of solvent (water) was taken in round bottom flask and the set up was run for approximately 8 hours and the extract was obtained. The obtained clove extract was stored under cold temperature.

PHYTOCHEMICAL ANALYSIS OF SAMPLE:

Phytochemical analysis is a type of Qualitative analysis is very essential to identify the phytochemical constituents present in medicinal plants. The medicinal value of plants is due to the presence of particular bioactive constituents such as flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

PHYTOCHEMICALS	OBSERVATION	INTERFERENCE
PHENOLIC COMPOUNDS (A)Gelatin Test	White precipitate	+
(B)Ferric chloride Test	Dark green colour	+
ALKALOIDS (A) Dangendroff's Test	Orange colour	+
(B) Mayer'Test	Creamy	_

	precipitate	
SAPONINS	No form formation	–
FLAVANOIDS	Bright yellow colour	+
GLYCOSIDES	No reddish brown colour	–
TANNINS	Dark blue colour	+
TERPENOIDS	Formation of layer	+

RESULTS FOR PHYTOCHEMICAL ANALYSIS

TOTAL PHENOL CONTENT DETERMINATION:

Total phenol content was expressed as mg gallic acid equivalent (mg GAE)/100 g sample dry weight.

Total Phenol content (TPC) 12.3 ± 0.5mg

ANTI MICROBIAL ACTIVIY OF Syzygium aromaticum:

The agar well diffusion assay by which the antimicrobial activity of S.aromaticum was confirmed. The plates were incubated at room temperature for 24 hours and the results was viewed and analysed, thus the inhibition zone confirms the antibacterial activity of S.aromaticum.

75µl concentration of S.aromaticum extract showed high zone of inhibition against four spices *MRSA , E.coli, Klebsella Pneumonia Enterococcus.*

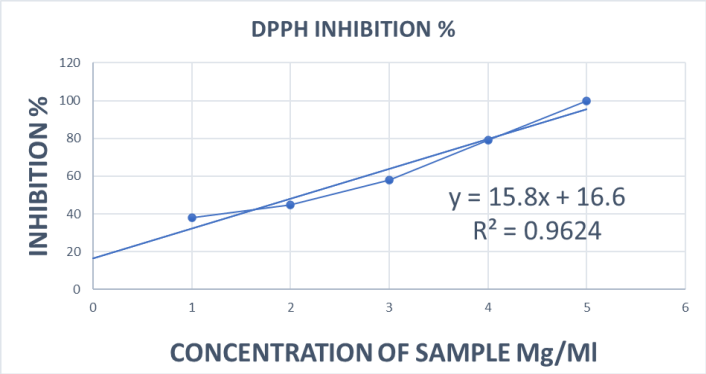
ANTI OXIDANT ACTIVITY OF *Syzygium aromaticum:*

DPPH ASSAY:

The Anti-Oxidant assay was performed by 2,2-diphenyl-1-picrylhydarzyl (DPPH) assay methodology. The absorbance was measured at 517nm.

S.No	VOLUME OF S.aromaticum EXTRACT(µl)	CONCENTRATION OF S.aromaticum EXTRACT (mg/ml)	OD AT 517 nm	INHIBITION %	IC50 Mg/Ml
1	Control	0	0.864	-	-
2	200	1	0.637	38	1.35
3	400	2	0.594	45	1.79
4	600	3	0.547	58	2.62
5	800	4	0.481	79	3.94
6	1000	5	0.432	100	5.27

Absorbance values for anti-oxidant activity of *S.aromaticum* by DPPH Assay.



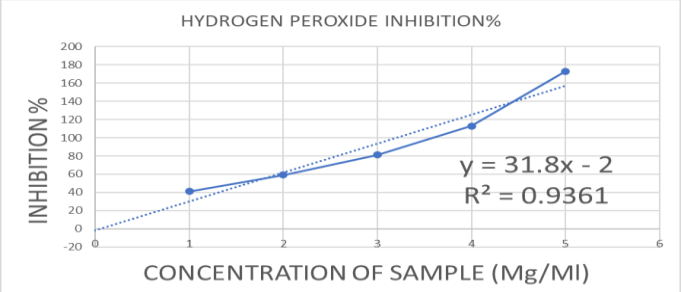
Represent the concentration of S.aromaticum extract vs DPPH inhibition %

HYDROGEN PEROXIDE ASSAY:

The Anti-oxidant assay was performed by hydrogen peroxide (H_2O_2). The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. The absorbance was measured at 230nm.

S.No	VOLUME OF S.aromaticum EXTRACT(µl)	CONCENTRATION OF S.aromaticum EXTRACT (mg/ml)	OD AT 230 nm	INHIBITION %	IC50 Mg/Ml
1	Control	0	0.693	-	-
2	200	1	0.491	41	1.35
3	400	2	0.437	59	1.91
4	600	3	0.382	81	2.61
5	800	4	0.326	113	3.61
6	1000	5	0.249	178	5.66

Absorbance values for anti-oxidant activity of S.aromaticum by Hydrogen Peroxide Assay.



Represent the concentration of S.aromaticum extract vs Hydrogen Peroxide inhibition %.

EVALUATION OF CHICKEN TREATED WITH EXTRACT:

The colonies growth was observed and analyzed.

Treatment Group	Days Of StoragE(Cfu/ml)				
	0	2	4	6	8
Control	2.16±0.02	2.85±0.02	3.15±0.03	4.43±0.07	5.47±0.04
Clove fermented	1.40±0.05	2.38±0.06	2.47±0.04	3.74±0.05	5.16±0.04

Microbial load of fermented and unfermented meat.

4. DISCUSSION:

The study was performed analyse the biological properties of clove extract against food spoilage microbes. The clove buds itself possess certain biological properties such as, Antibacterial, Antioxidant and Antiinflammatory activities. Thus the clove buds can be effectively used against food spoilage that was due to lipid oxidation etc,. The sample used was Syzygium aromaticum, which is commonly used spice. The extract was prepared by using Soxhlet extraction.

The plant extract was obtained by using water as solvent. The water was chosen as solvent, because it has the ability to extract maximum phytochemicals/ bioactive components from the sample. The extract were undergone phytochemical analysis and maximum phytochemicals were present.

The Total phenol content (TPC) was determined. The phenol content was major compound with helps to increase the shelf life time of meat, the sample also shows maximum antimicrobial activity against MRSA, E.coli, Enterococcus, Klebsella pneumonia and also has antioxidant activity determined by DPPH assay and Hydrogen Peroxide assay.

Therefore, the clove extracted was treated with chicken which has Bioactive compounds, and has the ability to control the microbial load in fermented chicken and act as natural preservative.

5. SUMMARY AND CONCLUSION:

The Syzygium aromaticum was a most common spice, and healthy because of its bioactive compounds and the presence of Antioxidant and Antimicrobial activity. S.aromaticum has an effective antimicrobial activity and this shows that it can be used as an natural preservative and decrease the microbial load of food products. The antioxidant activity of S.aromaticum is determined and it inhibits the oxidation which will also has controlled microbes in foods. The presence of phenolic compounds will also inhibit the spoilage of food products. Thus clove extract can be used as an food biopreservative for chicken and various food products. The antioxidant activity of phenolic compounds, which depends on the structure, consists of their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations. Natural antioxidants have been employed for meat preservation obtaining a significant delay of lipid oxidation, discoloration as well as microbial growth, thus extending meat lifetime. The results revealed the potential of clove as a natural biopreservative in meat .

6. REFERENCES

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