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STUDIES ON PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF *SYZYGIUM AROMATICUM* L.

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ABSTRACT

Plants are the source of inspiration for novel compounds. When a plant is preferred as 'medicinal' it is implied that the plant contains chemical substances which can be used for therapeutic purpose or it is a precursor for synthesis of useful drugs. The flower buds of clove have many medicinal properties like antiviral, antimicrobial, hypertensive aphrodisiac, carminative and anesthetic. In the present study, the proximate content, qualitative, quantitative, phytochemicals and antioxidant properties of *S. aromaticum* were analyzed by using standard methods. This investigation reveals that the phytochemicals such as alkaloids, saponins, flavonoids, tannins, terpenoids and anthroquinone were identified. Hence, the flower buds of *S. aromaticum* exhibit more bioactivities such as antibacterial, Antifungal and antioxidant activities were performed effectively.

KEYWORDS: *Syzygium aromaticum,* therapeutic, phytochemicals, antibacterial, antifungal and antioxidant.

INTRODUCTION

Plants are the nature's gift to mankind and from the very beginning of time plants have been used as an important source of food and medicine.[1] India has a rich tradition of plantbased knowledge on healthcare. According to World Health Organization (WHO) medicinal plants are those plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs.[2]

When a plant is preferred as "medicinal", it is implied that the assumed plant which, in one or more of its appendage, contains chemical substances that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs.[3,4] In this connection *Syzygium aromaticum* L. was selected as common traditional plant for their antimicrobial, anti-hyperglycemic and oxidative stress inhibitory effect. *S. aromaticum* is locally known as "lobongo" lavanga (Sanskrit) or laung, (Family- Myrtaceae) is a bushy, evergreen tree with a medium-sized crown, growing 8-20 meters tall native to the Maluku Islands in Indonesia, and are commonly used as a spice, throughout the year it commercially harvested in Bangladesh, Indonesia, India, Madagascar, Zanzibar, Pakistan, Sri Lanka, and Tanzania.

Its vast range of pharmacological activities has been well-researched and includes the treatment of analgesic, anesthetic effects.[5,6] In the present study, proximate content, qualitative and quantitative phytochemicals, antimicrobial activity and antioxidant properties of the flower buds of *S. aromaticum* were analysed.

MATERIALS AND METHODS

Collection of sample

The flower buds of *Syzigium aromaticum* were collected from the Thanjavur District, Tamil Nadu (Plate-1).

Systematic Position

Class : Dicotyledons
Order : Myrtales
Family : Myrtaceae
Genus : *Syzygium*
Species : *aromaticum*

Plant Description

The tree of clove, *Syzygium aromaticum* is evergreen tree. Then they height are 20 cm. It's stem is erect and it has gray bark. It's leaves until 12 cm long, oval- lanceolate or single, very aromatic, glossy green and leathery, like those of the sweet bay. The flowers are arranged in inflorescence in panicles at the end of young branches. They have four petals yellow rose and several pistils. Fruits small and elongated dark red berries.

Preparation of sample

The collected samples were air dried. After air dried the sample was ground in grinding machine made for the laboratory. Exposure direct sunlight was avoided to prevent the loss of active components. These powdered materials were used for further analysis.

Analysis of proximate content of *S. aromaticum*

Determination of moisture

Moisture was determined by oven drying method. 1.5 g of well-mixed sample was accurately weighed in clean, dried crucible (W1). The crucible was allowed in an oven at 100-105 C for 6-12 h o until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 min to cool. After cooling it was weighed again (W2). The percent moisture was calculated.

$$(\%)\ \text{Moisture} = \frac{W1-W2}{\text{wt of sample}} \times 100$$

Where,

W1 = Initial weight of crucible + Sample

W2 = Final weight of crucible + Sample

Determination of Crude Fiber (CF)

About 2 gm. of the dried, fat-free sample was taken into a 600 ml beaker. 200 ml of hot sulphuric acid was added and the beaker was placed under the condenser and boiled gently for exactly 30 min. Distilled water was used in order to maintain volume and to wash down particles sticking to the sides. Filtered through Whatman No. 541 paper in a buchner funnel, using suction. Washing with boiling water has been provided. Residue was transferred back to the beaker and 200 ml hot sodium hydroxide solution was added. Replaced under the condenser and again brought to boiling within 1 min. After boiling for exactly 30 min, it was filtered through a porous crucible and washed with boiling water; 1% hydrochloric acid and then again with boiling water. Mashing was given twice with alcohol or acetone, dried overnight at 100°C, cooled and weighed. Ashed at 500°C for 3 hours, cooled and weighed. The weight of fiber was calculated by the difference in weight.

Determination of nitrogen free extracts (NFE)

Nitrogen free extract of a sample was determined by difference after the analysis has been completed for ash, crude fiber, crude fat and crude protein.

Calculation

NFE on dry basis = 100%-(% ash on dry basis+% crude fiber on dry basis+% crude fat on dry basis+% protein on dry basis.

Acid Soluble and Insoluble Ash

The residue obtained from the ash determination was used. Boiled with 20 ml 50% HCl being careful to avoid spattering filtered through ash less filter paper and washed with hot water until acidfree. Filter paper and residue both was transferred into a dry porcelain dish and placed in a muffle furnace at 600 ˚C for 2 hours.

Calculation

$$\text{Ash Insoluble ash (\%)} = \frac{\text{Wt. of acid treated ash}}{\text{Wt. of sample}} \times 100$$

Determination of Ash

Clean crucibles were placed under a muffle furnace at 600 °C for one hour. Crucibles were moved from the furnace to a desiccator and cooled to room temperature. These crucibles were weighed quickly to prevent moisture absorption. 2.0 gm. of sample was added into dried silica crucibles. Placed in a muffle furnace and the temperature were held at 600˚C for 6 h ours. The crucibles were taken to a desiccator and cooled to room temperature. After cooling crucibles, were weighed as quickly as possible to prevent moisture absorption. The ash sample was saved for mineral determinations.

Calculation

$$\text{Ash (\%) on partial dry or as fed basis} = \frac{\text{Wt. of ash}}{\text{Wt. of sample}} \times 100$$

$$\text{Adjusting to dry basis} = \frac{\text{ash \% on as sample}}{\text{dry matter \% of as sample}} \times 100$$

Preparation of plant extract

The dried flower buds of *S. aromaticum* were pounded to powdered and then subsequently sieved. 50g of the powdered *S. aromaticum* was weighed into bottle and 500ml of distilled water was added. This was to carry out in aqueous extract. The plant material was soaked in the solvent for 24hrs and then filtered. The filtered was concentrated to get the crude extract from which different concentrations were prepared and stored at 4^{o} C.

Qualitative and Quantitative Phytochemical Analysis

Preliminary phytochemical analysis (qualitative and quantitative) was carried out as per standard methods.[7]

Determination of antimicrobial activity[8]

Test microorganisms

The following bacterial and fungal strains were used for the screening of antimicrobial activity. All the microbial strains of human pathogens used were procured from IMTECH, Chandigarh and procured microbes are the Gram negative bacteria, *viz. Aeromonas* sp., *Lactobacillus* sp., *Escherichia coli* and the Gram positive bacteria, *Proteus vulgaris* and *Staphylococcus aureus* and fungi *viz., Aspergillus flavus, A. niger, A. terreus, Trichoderma* sp. and *Pencillium* sp. were selected for this study.

Media used

Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used for testing the antibacterial and antifungal activity.

Agar well – diffusion method

Agar well – diffusion method was followed to determine the antimicrobial activity. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old – broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About 100µl of different solvent leaf extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre – incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions the plates were incubated in an upright position at 37˚C ± 2˚C for 24 h for bacterial pathogens and 28˚C ± 2˚C for fungi. The organic solvents alone were acted as a negative control. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. The effect of plant extract was compared with standard antibiotics. Triplicates were maintained and the average values were recorded for antimicrobial activity.

Antioxidant assay

Reducing power assay[9]

The reducing power of the aqueous extract was determined according to this method. One ml of the leaf extract containing (0.2 – 0.8 microgram) in 1ml of the deionized water mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferrocyande (1%). The mixture was incubated at 50ºC for 20 minutes. 2.5ml of TCA (10%) and centrifuged at 3000 rpm. The upper layer of the solution was mixed with 2.5ml distilled water and FeCl3 (0.5ml, 0.1%). The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated the higher reducing power. The absorbance compared with the standard ascorbic acid (concentration 20 µg).

The percent increase in reducing power was calculated using the following equation,

$$\text{Increase in reducing power (\%)} = \frac{\text{A test - A std}}{\text{A std}} \times 100$$

Hydrogen peroxide

Scavenging activity of H_2O_2 radical activity[10]

The H_2O_2 scavenging of the fish samples was determined according to this method. A solution of H_2O_2 (40 mM) was prepared in PBS (pH 7.4) and concentration was determined spectrophotometrically (Gene Quant 1300 UV-Vis) at 230 nm. The leaf extracts(0.2 – 0.8 microgram) were added to a H_2O_2 solution (0.6 ml, 40 mM) and incubated at room temperature for 10 minutes, the absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing the leaf extracts without H_2O_2. Ascorbic acid was used as standard (concentration 20µg). The percentage scavenging of H_2O_2 was calculated using the following equation:

Scavenging activity of H_2O_2 radical activity= [(Abs std – Abs sample) / Abs std] × 100.

Thiobarbitaric Acid (TBA) Method

TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100ºC), melonaldehyde binds with TBA to form a red complex that can measured at 532 nm. 2 ml of 20% Trichloroacetic acid and 2 ml of 0.67% TBA solutions were added to 2 ml of the mixtures containing the sample (0.2 – 0.8 microgram) prepared in the FTC (Ferric thiocyanate) method. This mixture was kept in water bath (100ºC) for 10minutes and after cooling to room temperature, was centrifuged at 3000 rpm for 20

minutes. Antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay. Malondialdehyde used as a standard (concentration of 20µg). The percentage of antioxidant activity was calculated by following formula for TBA.

$$\text{Percentage of activity} = \frac{\text{Absorbance of (Control –Test)}}{\text{Absorbance Control}} \times 100$$

RESULTS

The qualitative phytochemical analysis of *Syzygium aromaticum* L. with aqueous extract was determined. The results revealed the presence of pharmacologically active compounds such as alkaloids, saponins, flavonoids, tannins, phenol, steroid, terpenoids, protein, anthroquinone and reducing sugars were tested. Among the tested phytochemical like alkaloids, saponins, flavonoids, tannins, terpenoids and anthroquinone were represented respectively (Table-1 and Plate-1). Whereas in the case of quantitatively analysed phytochemicals like alkaloids (1.08 mg/ml), flavonoids (1.58 mg/ml), saponins (1.15 mg/ml) and tannins (1.45 mg/ml) were recorded in Table-2.

The antibacterial activity of the flower buds of *S. aromaticum* aqueous extract was assessed by agar plate diffusion technique. Among all the five bacterial strains, *Lactobacillus* sp. was shown maximum potential of antibacterial activities observed as 22, 23, 25 and 30mm zone of inhibition measured with the concentration of 25, 50, 75 and 100mg of *S. aromaticum* was respectively. Whereas the minimum zone of inhibition was observed in *Aeromonas* sp. (Table-3).

The antifungal effect of *S. aromaticum* with aqueous extract was evaluated *in vitro* against some fungi such as *Aspergillus flavus, A.niger, A.terreus, Penicillium* sp. and *Trichoderma* sp. with some concentration of 25, 50, 75 and 100 mg of test plant extract was analyzed respectively. All the fungal strains showed maximum zone of inhibition with increasing the concentration of plant extract (Table-4).

The determination of antioxidant potential of *S. aromaticum* has maximum (75%) of activity by the plant. It was 32, 38, 43, 51 and 59% of antioxidant activity with different concentration of 0.2, 0.4, 0.6, 0.8 and 1.0% of the extract recorded respectively by reducing power assay method (Table 5-1). Whereas hydrogen peroxide assay also maximum antioxidant activity was 37, 44, 53, 69 and 74% with 0.2, 0.4, 0.6, 0.8 and 1.0% of concentration of the extract recorded respectively (Table 5-2). According to the thiobarbutric

acid assay was 29, 32, 44, 49 and 54% with the concentration of 0.2, 0.4, 0.6, 0.8 and 1.0% was observed respectively (Table 5-3). The results proved that the presence of biological and pharmacologically active compounds were main responsible for antibacterial, antifungal and antioxidant activities.

Plate 1: Flower buds of *Syzygium aromaticum* L.

Table 1: Qualitative phytochemical analysis of *Syzygium aromaticum*.

S. No.	Name of the compounds	Inference
1	Alkaloids	+
2	Saponin	++
3	Flavonoids	+++
4	Tanin	++
5	Phenol	_
6	Steroid	_
7	Terpenoids	+
8	Protein	+
9	Anthroquinone	+
10	Reducing sugar	_

+++ (strongly present), ++ (moderate), + (present), -- (absent)

Table 2: Quantitative phytochemical analysis of *Syzygium aromaticum*.

S. No.	Name of compounds	Solvent
1	Alkaloids	1.08
2	Flavonoids	1.58
3	Saponin	1.15
4	Tannin	1.45

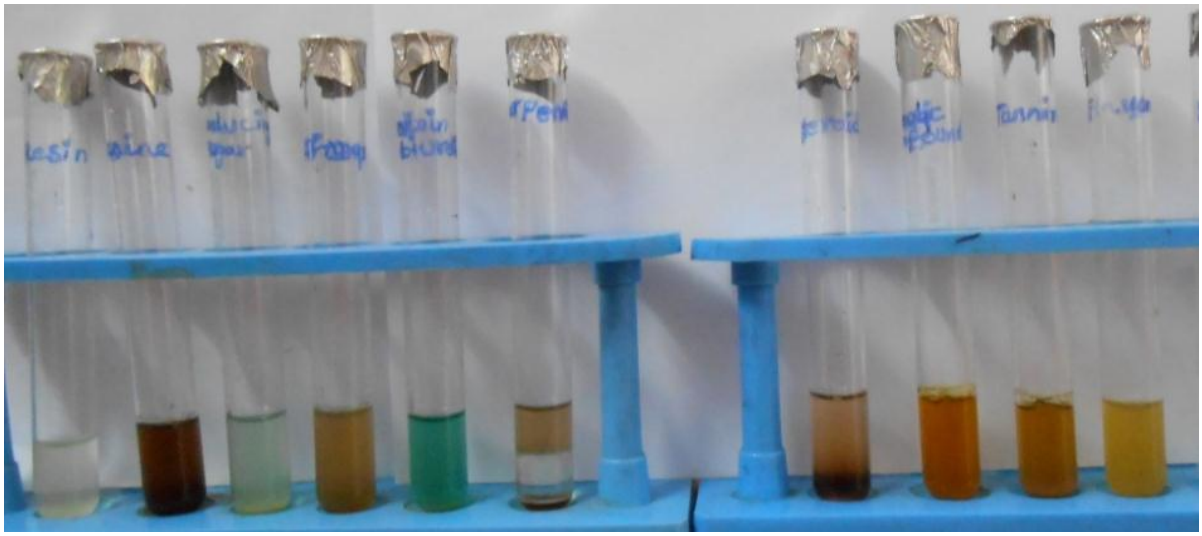


Plate 2: Qualitative phytochemical analysis of *Syzygium aromaticum.*

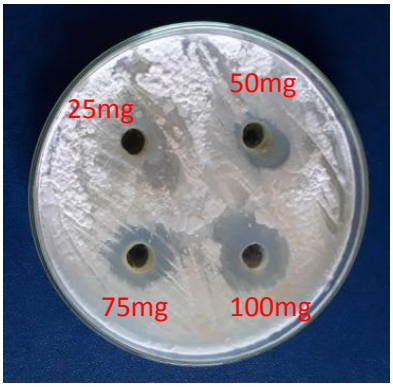
Table 3: Antibacterial activity of *Syzygium aromaticum* against some clinical bacteria.

S.No	Name of the Bacteria	Zone of inhibition(mm)			
		25mg	50mg	75mg	100mg
1	*Aeromonas* sp	05	08	10	15
2	*E.coli*	05	15	17	20
3	*Lactobacillus* sp	22	23	25	30
4	*Proteus vulgaris*	10	15	25	35
5	*Staphylococcus aureus*	10	15	19	20

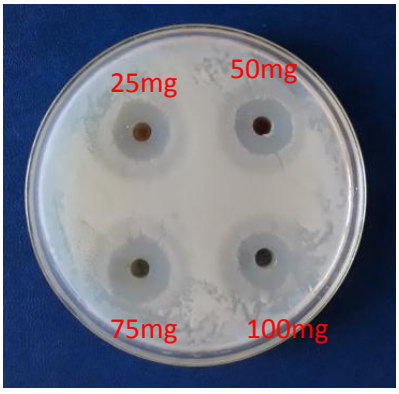
Table 4: Efficacy of Antifungal activity of *Syzygium aromatium* against some fungi.

S. No.	Name of the fungi	Zone of inhibition (mm)			
		25 mg	50 mg	75 mg	100 mg
1	*Aspergillus flavus*	15	15	20	20
2	*A. niger*	9	15	20	25
3	*A. terreus*	10	12	16	20
4	*Penicillium* sp.	10	15	15	20
5	*Trichoderma* sp.	10	10	15	20

Plate 3: Antibacterial activity of *S. aromaticum* against some clinical bacteria.



***Aeromonas* sp.**



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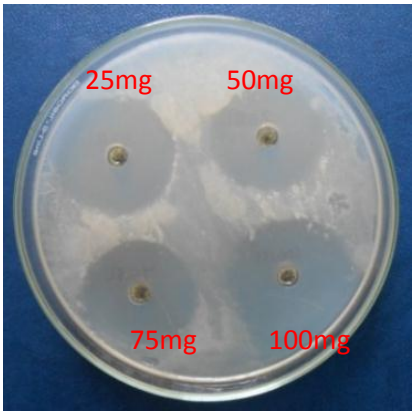
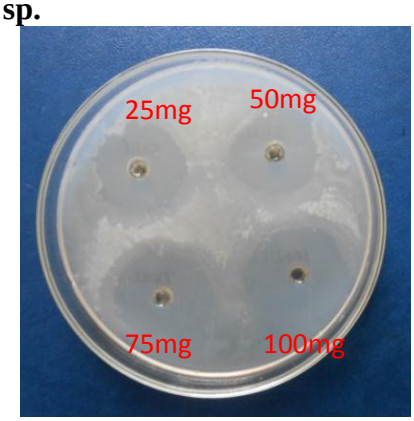
***Lactobacillus* sp.**

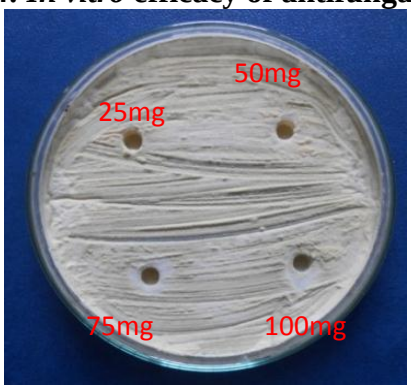


Proteus vulgaris

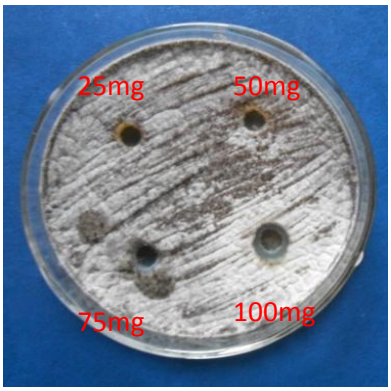


Staphylococcus aureus

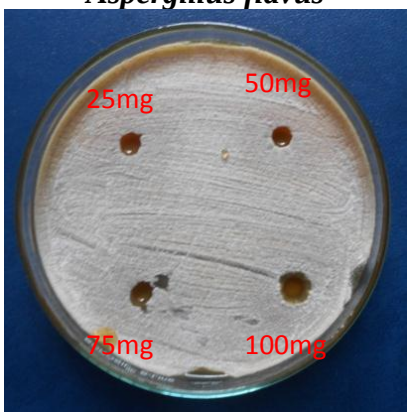
Plate 4: *In vitro* efficacy of antifungal activity of *S. aromaticum*.



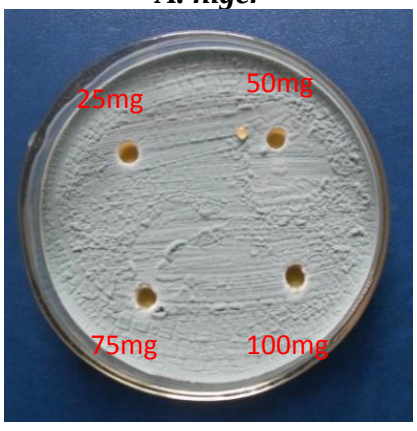
Aspergillus flavus



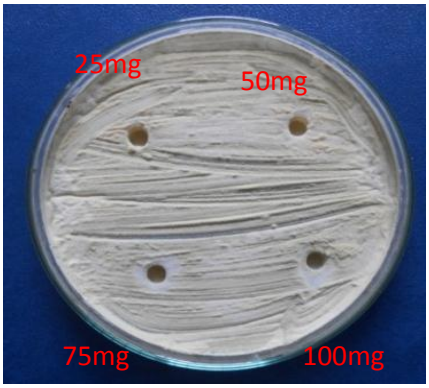
A. niger



A. terreus



***Penicillium* sp.**



***Trichoderma* sp.**

Table 5: Determination of Antioxidant activity of *Syzygium aromatcmum.*

1. Reducing power assay.

S. No.	Concentration of *Syzygium aromaticum* (%)	Antioxidant activity (%)
1	0.2	32
2	0.4	38
3	0.6	43
4	0.8	51
5	1.0	59

2. Hydrogen peroxide assay.

S. No.	Concentration of *Syzygium aromaticum* (%)	Antioxidant activity (%)
1	0.2	37
2	0.4	44
3	0.6	53
4	0.8	69
5	1.0	74

3. Thiobarbitaric acid method.

S. No.	Concentration of *Syzygium aromaticum* (%)	Antioxidant activity (%)
1	0.2	29
2	0.4	32
3	0.6	44
4	0.8	49
5	1.0	54

DISCUSSION

The world population is relying on traditional medicines with traditional therapies of the plant system performed. In the current investigation suggested that the entitled on *Syzygium aromaticum* were determined. Proximate analysis revealed high amount of carbohydrate content ranged from 38.46-66.54%. Moisture content was found to be highest in *Eclipta alba*

(89.1%) while protein was higher in *Houttuynia cordata* (19.68%). Fat content was relatively less and fiber content ranged from 9.4 - 23.52%. These vegetables were found to be rich sources of macroelements as well as trace minerals. Potassium was the most abundant macroelement ranging from 6240.0-14570.0 mg/kg, followed by sodium, calcium and magnesium. Among the trace elements Iron was highest (252.8-712.9mg/kg), followed by zinc, manganese and Copper. The results demonstrated that these 5 selected underutilized medicinal plants have great nutritional significance.[11] In the present research revealed that the plant *S. aromaticum* has potential source by phytochemical active compounds were represented and analysed. It was alkaloids, saponins, flavonoids, phenol, tannins, steroids, terpenoid, protein, anthroquinone and reducing sugars recorded respectively. whereas quantitatively alkaloids, flavonoids, saponins and tannins was 1.08, 1.58, 1.15 and 1.45 mg/ml recorded respectively.

Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties. They are non essential nutrients but the plants that produce these chemicals protect humans against diseases. The phytochemical studies shows that the ethanolic leaf extract is rich in flavonoid, phenols, tannins, alkaloids, steroids and terpenoids. These secondary metabolites act as an effective antimicrobial substance against wide range of microorganisms.[12] Terpenoids acts as bronchodilator in humans and exhibit anti-inflammatory, antibacterial and antibiotic properties. Alkaloids possess numerous functions, among them the most important are their analgesic, antispasmodic and bactericidal effects.[13] Flavonoids, another constituent of leaf extract of *Costus speciosus* exhibited a wide range of biological activity like antimicrobial, anti-inflammatory, antiallergic and antioxidant properties.[14] Polyphenolic compounds like flavonoids and tannins are known antioxidant and possess organ protective functions. Flavonoids are potent free radical scavengers, which prevent oxidative cell damage, have anticancer activity.[15]

SUMMARY AND CONCLUSION

The plant products having medicinal properties are commonly known as medicinal plants. These medicinal plants are known to possess various phytochemicals which exhibit more bioactivities such as antibacterial, antifungal, antioxidant activities were performed. Some of the phytochemical compounds were represented such as alkaloids, saponins, flavonoids, tannins, terpenoids and anthroquinone were identified from the *S.aromaticum* plant some of few phytochemical are quantitatively recognized from the study. With regarding

antimicrobial activities were planned to suppress the activities of pathogens control measures developed. The maximum antibacterial activities of *S. aromaticum* against *Proteus vulgaris* followed by all the bacterium performed as well as the antifungal activities was the maximum at *A. niger* followed by other fungi *A. flavus, A. terreus*, *Penicillium* sp. and *Trichoderma* sp. observed respectively. According to the antioxidant properties of *S. aromaticum* were investigated. The three methods the hydrogen peroxide assay was excellent antioxidant properties when compared with other activity of reducing power assay and thiobarbutaric acid method. However, the *S. aromaticum* has extraordinary biological and pharmacological activities were analyzed and investigated from the study.

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