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Syzygium aromaticum Methanol Extract [SAME] phytochemical analysis and its role on pathogenic organisms

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Abstract

The aim of this work is to explore the possible medical applications of *Syzygium aromaticum* Methanol Extract [SAME]. SAME has an abundance of macro and micronutrients, including phytosterol, phenolic compounds, saponins, flavonoids, and more, based on the results of the initial screening assay. GC-MS and HPLC analyses, which yielded chromatograms with 11 and 6 peaks, respectively, demonstrating the presence of various phytochemicals, also confirmed their presence. Furthermore, minerals like zinc, manganese, iron, boron, and aluminum are present in the SAME extract. In addition to the tests mentioned above, we are fortunate to have found that SAME's non-toxic property keeps it from cleaving packed red blood cells in *in vitro* tests. SAME surprisingly possesses antibacterial qualities against gram positive bacteria (S. *aureus*) and gram negative bacteria (E. *coli*). Amoxicillin used as a positive control. The minimum inhibitory concentration for both the organisms is 1.4 and 0.8 value respectively.

Keywords: *Syzygium aromaticum* Methanol Extract [SAME], GC-MS, RP-HPLC, Anti-bacterial and Non-toxic property

Introduction

The traditional medical approach, which relies on the application of herbal remedies, is still very much a part of the healthcare system. Medicinal plants have become more widely accepted in recent decades [1]. Because it is believed that these plants' natural products are more effective and have fewer side effects than their synthetic counterparts. Approximately 80% of people on the planet now receive the majority of their primary healthcare from traditional medicine [2]. Many herbal plants are used in food preservation and embalming, and their pharmacological properties include bactericidal, virucidal, and fungicidal properties. They also have anti-inflammatory, antimicrobial, spasmolytic, sedative, analgesic, and local anesthetic properties [3]. It has been reported that the phytoconstituent of many plant species, including glycosides, saponins, flavonoids, steroids, tannins, and alkaloids, have pharmacological properties [4]. Clove, or Syzygium (S.) aromaticum, is a dried flower bud of the Myrtaceae family that is native to Indonesia's Maluku islands but has recently been farmed in various locations across the world [5]. The leaves and buds of the clove tree make up its commercial portion.

And four years after plantation, flowering bud production starts [6]. Then, when they are in the pre-flowering stage, they are harvested manually or with the aid of a natural phytohormone [7]. Interestingly, cloves are one of the spices that can potentially be used as preservatives in many foods, especially in meat processing, to replace chemical preservatives because of their antioxidant and antimicrobial properties [8]. They are also commercially used for many medicinal purposes and in the perfume industry. The presence of numerous chemical constituents in high concentrations with antioxidant activity is thought to be

responsible for clove's effective role in the inhibition of various degenerative diseases [9]. Clove essential oil (CEO) has long been used to treat toothaches, tooth infections, burns, and wounds. It is also used as a pain reliever in dental care [10].

Materials and Methods

All the chemicals used were of analytical grade. Microbial cultures were purchased from MTCC.

Preparation of SAME: *Syzygium Aromaticum* was purchased from local market and subjected for Soxhlet extraction method using Methanol solvent. The finally obtained extract was termed as *Syzygium Aromaticum* Methanol Extract and it utilized for further assays.

Preliminary phytochemical screening of SAME

SAME was screened for terpenoids, phytosterol, tannin, phenolic, glycoside, Saponin, flavonoid, carbohydrates, proteins, steroids and alkaloids [11].

RP-HPLC analysis of SAME

SAME was subjected to RP-HPLC using C_{18} column (150mm×3mm, particle size 2.7µm) with VWD detector in Agilent 1260-infinity II. The column was pre-equilibrated with HPLC water and Acetonitrile and sample was eluted at the flow rate of 1ml/min in linear gradient mode [12].

GC-MS analysis of SAME

SAME was analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Impact Ionization Total Ion Chromatography (EITIC) mode with capillary column (30m lengthX0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.999%) gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 2µl. Split ratio of 10:1, temperature program was set as follows, injector temperature 350 °C; Auxiliary temperature 250 °C, oven temperature initially 50 °C (4min hold) with an increase in temperature of 10 °C/min to 150 °C (4min hold), thereafter 20 °C/min to 200 °C (4min hold), 25 °C/min ramp to 250 °C (4 min hold), 30 °C/min ramp to 280 °C (4 min hold). Total run time 35.5 min. Mass spectrum was taken at 70ev; a scan interval of 2.92s [13].

Direct hemolytic activity of SAME

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1mL of this suspension was incubated independently with the various concentrations of SAME (100µL & 200µL) for 1hr at 37 °C. The reaction was terminated by adding 9mL of ice cold PBS and centrifuged at 1000g for 10min at 37 °C [14]. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to the addition of water (positive control), whereas PBS served as negative control.

Antimicrobial assay of SAME

The bacterial cultures (*E. coli* and *S. aureus*) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient broth swabs were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates (Muller Hinton Agar) evenly with the help of a swab. Wells were made on the solidified agar (MHA) using a cork borer. The test solution was made by dissolving 50mg of SAME in 1.0mL of water to get 50mg/mL concentration followed by sonication for 2min. The 100µL of this test solution containing 5mg of SAME was added into the respective wells. The standard antibiotic drug Amoxycillin was kept as positive control and tested against both the pathogens. These plates were incubated at 37 °C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [15]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported.

ICP-OES analysis of SAME

SAME was analyzed in Agilent Make ICP-OES instrument, model number 5110. To evaluate the content of minerals in the extract, the samples were aspirated at 12 RPM pump speed, 25 seconds sample uptake time, 30 seconds of rinse time, 5 seconds, read time, 1.2 KW RF power, 15 seconds stabilization time, Axial viewing mode, 8mm viewing height, 0.7 L/Min nebulizer flow, 12 L/Min plasma flow, 0.75 L/Min Aux flow [16].

Results and Discussion

Chemical Characterization of SAME

SAME found to presence of phenolic compounds, flavonoids, phenolic compounds, saponins, phytosterol and etc., (Table 01). SAME shows the presence of several minerals such as aluminium, boron, barium, copper, iron, manganese, lead, zinc and etc., (Table 02).

Table 1: Phytochemical Analysis and Results

Sl. No.	Phytochemical Analysis	Results
1	Terpenoid	Absent
2	Phytosterol	Present
3	Tannin	Absent
4	Phenolic	Present
5	Glycoside	Absent
6	Saponin	Present
7	Flavonoid	Present
8	Carbohydrates	Absent
9	Proteins	Absent
10	Alkaloid	Absent
11	Steroids	Absent

Table 2: Name of the Metal and SAME in ppm

Sl. No.	Name of the Metal	SAME in ppm
1	Aluminium	0.95
2	Boron	0.24
3	Barium	0.15
4	Cadmium	0.00
5	Copper	0.05
6	Iron	2.31
7	Manganese	5.10
8	Molybdenum	0.01
9	Nickel	0.02
10	Lead	0.04
11	Zinc	0.40

RP-HPLC analysis of SAME

SAME elutes 6 peaks at different retention time in reverse phase HPLC chromatogram which is attached to Variable Wavelength Detector. Sample was eluted at 216nm at room temperature (Fig.01).

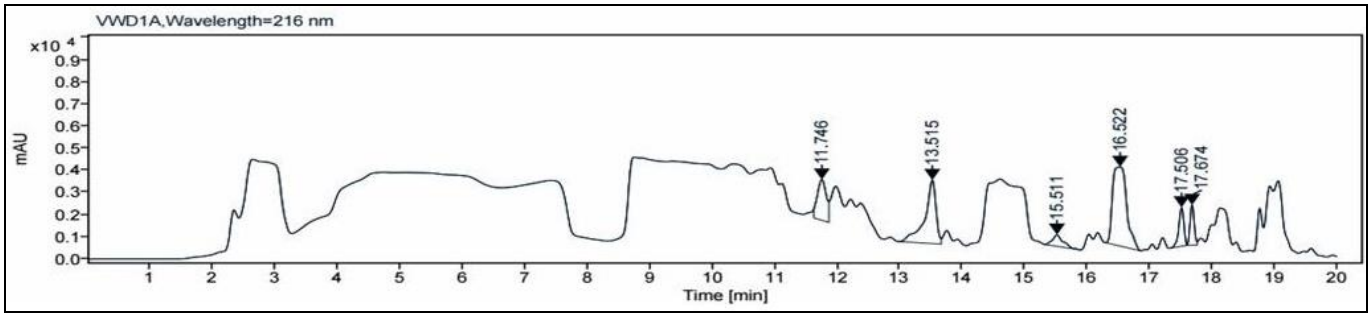

Fig 1: HPLC Chromatogram of SAME

GC–MS analysis of SAME

SAME elutes 11 peaks in GC-MS chromatogram at the retention time of 2.5, 13.2, 14.2, 18.2, 18.9, 19.5, 21.0, 21.8, 22.3, 23.9 and 26.6 respectively (Fig.02).

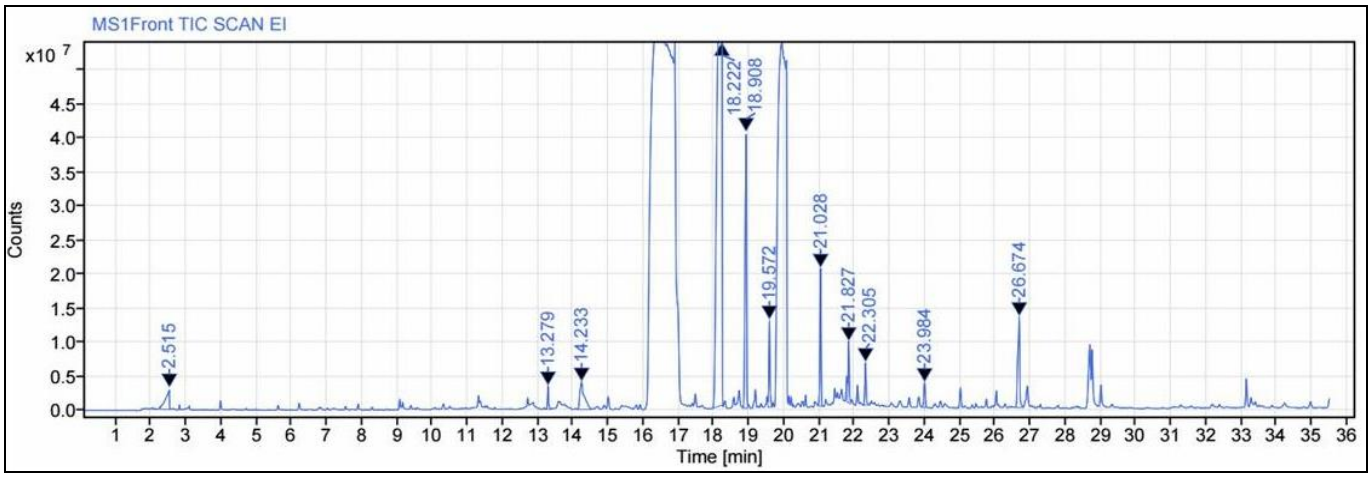

Fig 2: GC MS Chromatogram of SAME

Antimicrobial activity of SAME

SAME antimicrobial property was performed with both gram negative bacteria (*E. coli*) and gram positive bacteria (*S. aureus*). Astonishingly, SAME found to show zone of inhibition against both the bacteria (Fig.03).

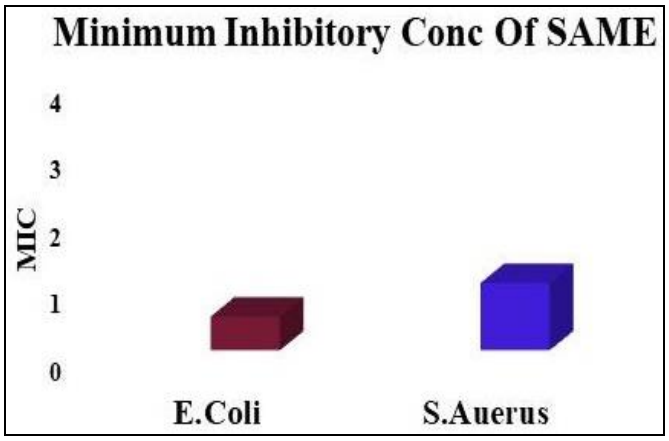

Fig 3: Antimicrobial Property of SAME

Moreover, SAME did not hydrolyze RBC suggested its nontoxic property (Fig.04).

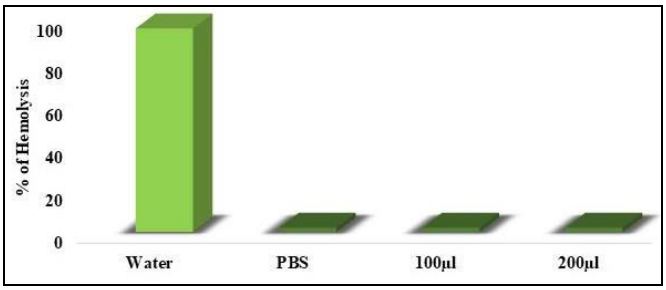

Fig 4: Haemolytic activity of SAME

Conclusion

The preliminary characterization of SAME and its antimicrobial activity against both gram positive and gram negative bacteria are presented in the study's conclusion.

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Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

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