Online at: https://researchtrendsjournal.com ISSN No: 2584-282X

INTERNATIONAL JOURNAL OF TRENDS IN EMERGING RESEARCH AND DEVELOPMENT

Volume 2; Issue 5; 2024; Page No. 01-05

Received: 02-08-2024
Accepted: 04-09-2024

Exploration of adulterants and total minerals in LAE, KAE, SAE and MAE

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DOI: https://doi.org/10.5281/zenodo.13751578

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Abstract

The aim of this work is to explore the adulterants and total minerals in LAE, KAE, SAE and MAE. HPLC and GC-MS chromatogram conforms the adulterants were present in the LAE, KAE, SAE and MAE as it elute 3, 4, 3, 3 peaks in HPLC and 10, 10, 7, 6 peaks in GC-MS respectively. In addition, LAE, KAE, SAE and MAE also withhold several minerals and heavy metals such as zinc, manganese, iron, aluminium, chromium, copper, nickel and strontium. Moreover, LAE, KAE, SAE and MAE possess non-toxic property as it was unable to cleave packed RBC in in-vitro study at the concentration of 200µg. Astonishingly; LAE, KAE, SAE and MAE exhibits anti-microbial property as it produce zone of inhibition in both gram negative bacteria *E. coli* and gram positive bacteria *S. aureus*. The obtained anti-bacterial results are demonstrated in Minimum Inhibitory Concentration (MIC) values for LAE, KAE, SAE and MAE.

Keywords: LAE, KAE, SAE, MAE, GC-MS, RP-HPLC, Anti-microbial and non-toxic property

Introduction

The demand for processed foods has increased dramatically as a result of dietary behavior changes, pushing aside traditional wholesome eating habits. Modern diets have been linked to non-communicable diseases (NCDs) worldwide [1]. According to the 2017 Global Burden of Disease (GBD) estimate, dietary risk factors were responsible for 11 million deaths and 255 million disability-adjusted life years (DALYs) [2]. The World Health Organization (WHO) has designated certain dietary components-trans fats and sodium/salt in processed foods, for example-as priority areas to promote population health and wellbeing in its 13th general program of work due to growing concerns about their negative health effects. Whatever their level of development, all nations exhibit shifting dietary habits [3]. But the patterns and influencing elements vary by nation. The expanding food processing industry's increased supply in response to the rising demand contributes to the evolving trends. As a result, there has been evidence of an increase in the burden of non-communicable diseases in Africa. Intriguingly, a recent meta-analysis highlighted the connection between diet-sensitive NCDs in Africa and food insecurity as a risk factor for metabolic disorders [4]. According to the WHO's NCD progress monitor, between 50% and 88% of deaths in seven small African countries were attributable to NCDs, pointing to unsustainable urbanization, changes in lifestyle, and the multiple burdens of disease [5]. According to the British Soft Drinks Association Annual Report 2016, soft drinks comprise carbonated beverages, still and juice drinks, bottled waters, fruit juices, dilutables, sports drinks, and energy drinks [6]. In the UK, soft drink consumption as a whole grew by 0.2% between 2010 and 2015, according to the British Soft Drinks Association Annual Report (2016) [7]. Compared to 13.2 billion liters in 2010, 13.3 billion liters of soft drinks were consumed in 2015. Of those, 58% were low-or no-calorie varieties (0–20 kcal per 100 ml) [8]. Certain soft drinks may be detrimental to people's general

health and dental health, especially in young people and adolescents [9]. Manufacturers and government organizations have taken steps to lessen the possible negative effects that sugar-filled soft drinks may have on teeth and overall health [10]. These include outlawing the sale of soft drinks in educational institutions, limiting the promotion of soft drinks, changing the ingredients in soft drinks, and levying taxes on soft drinks that contain sugar [11].

Materials and Methods

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

Preparation of LAE, KAE, SAE and MAE

We purchased packed snacks kurkure and lays with sprite and maza soft drinks for the research work from the local market. The analytes were prepared by acid digestion using Nitric acid and Hydrochloric acid mixture with hydrogen peroxide and the obtained extract was termed as KAE (Kurkure Acid Extract), LAE (Lays Acid Extract), SAE (Sprite Acid Extract) and MAE (Maza Acid Extract). The final obtained extracts were termed as LAE, KAE, SAE and MAE it utilized for further assays.

RP-HPLC analysis of LAE, KAE, SAE and MAE

LAE, KAE, SAE and MAE were 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 LAE, KAE, SAE and MAE

LAE, KAE, SAE and MAE were 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 length X0.25mm 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 LAE, KAE, SAE and MAE

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 LAE, KAE, SAE and MAE (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 LAE, KAE, SAE and MAE

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 LAE, KAE, SAE and MAE in 1.0mL of water to get 50mg/mL concentration followed by sonication for 2min. The 100µL of this test solution containing 5mg of LAE, KAE, SAE and MAE were 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 LAE, KAE, SAE and MAE

LAE, KAE, SAE and MAE were 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 LAE, KAE, SAE and MAE

LAE, KAE, SAE and MAE shows the presence of several minerals such as aluminium, copper, iron, manganese, lead, zinc and etc., (Table 01).

Table 1: Shows the presence of several minerals such as aluminium, copper, iron, manganese, lead, zinc and etc.

SL. NO.	Name of The Metal	LAE (PPm)	KAE (PPm)	SAE (PPm)	MAE (PPm)
01	Aluminium	0.71	0.69	0.69	0.23
02	Boron	0.00	0.00	0.00	0.00
03	Cadmium	0.00	0.00	0.01	0.00
04	Chromium	0.03	0.05	0.30	0.03
05	Copper	0.05	0.02	0.21	0.02
06	Iron	0.44	0.31	1.36	0.18
07	Manganese	0.08	0.07	0.11	0.03
08	Molybdenum	0.00	0.00	0.01	0.00
09	Nickel	0.04	0.02	0.50	0.01
10	Lead	0.01	0.01	0.03	0.00
11	Strontium	0.04	0.04	0.22	0.03
12	Zinc	0.36	0.51	0.58	0.06

RP-HPLC analysis of LAE, KAE, SAE and MAE

LAE, KAE, SAE and MAE elute 3, 4, 3 and
3 peaks respectively 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.01-04).

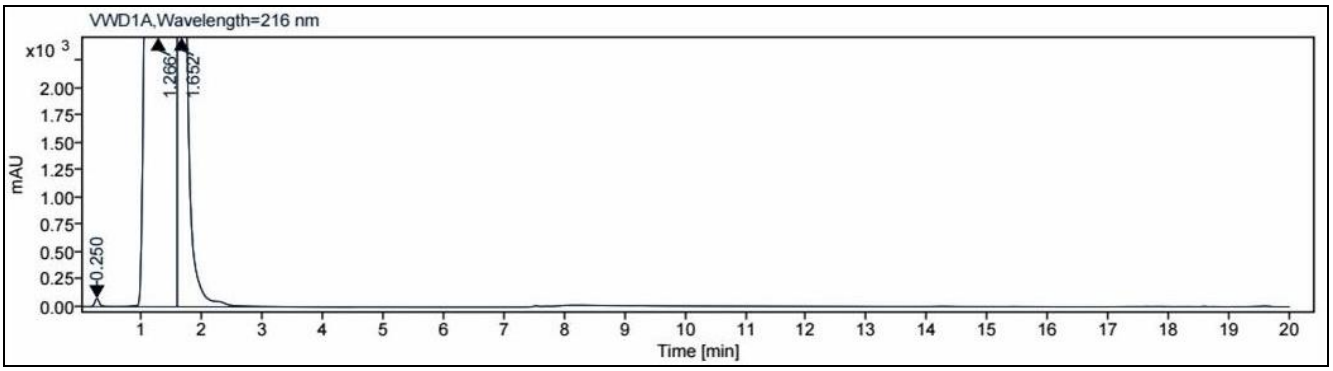

Fig 1: HPLC Chromatogram of LAE

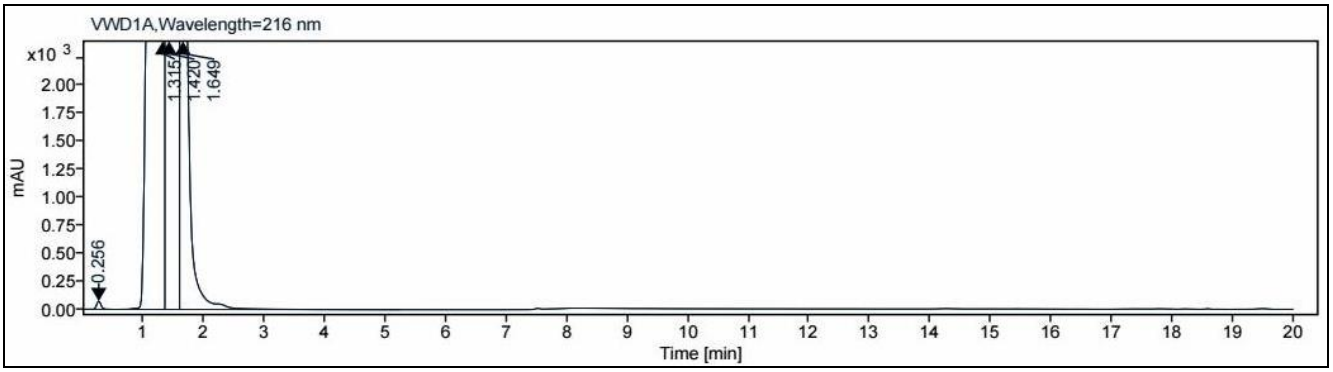

Fig 2: HPLC Chromatogram of KAE

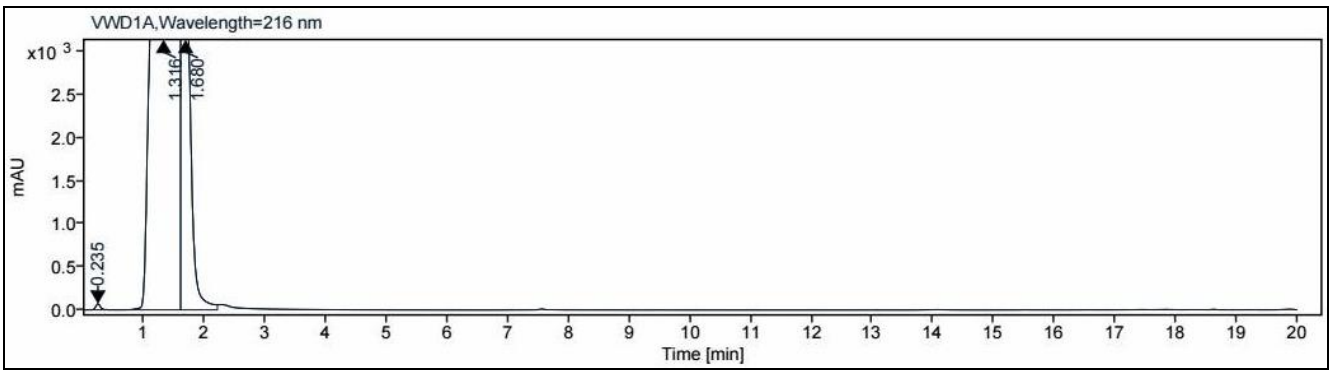

Fig 3: HPLC Chromatogram of SAE

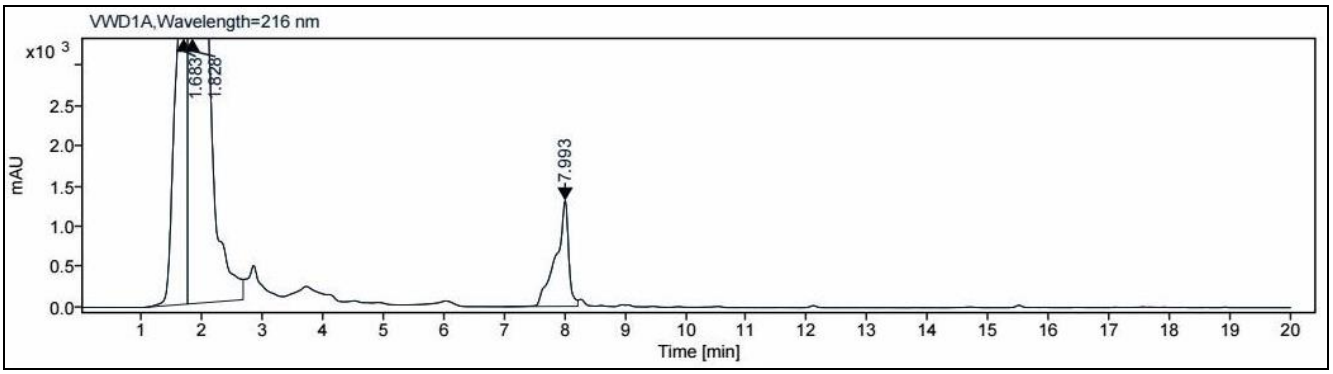

Fig 4: HPLC Chromatogram of MAE

GC–MS analysis of LAE, KAE, SAE and MAE

LAE, KAE, SAE and MAE elute 10, 10, 7 and 6 peaks respectively in GC-MS chromatogram at different retention time (Fig.05-08).

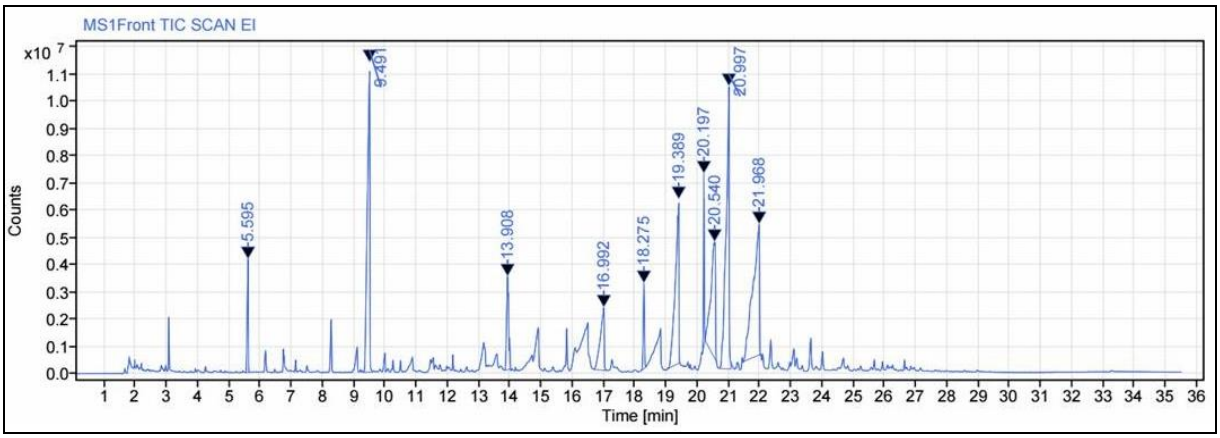

Fig 5: GC MS Chromatogram of LAE

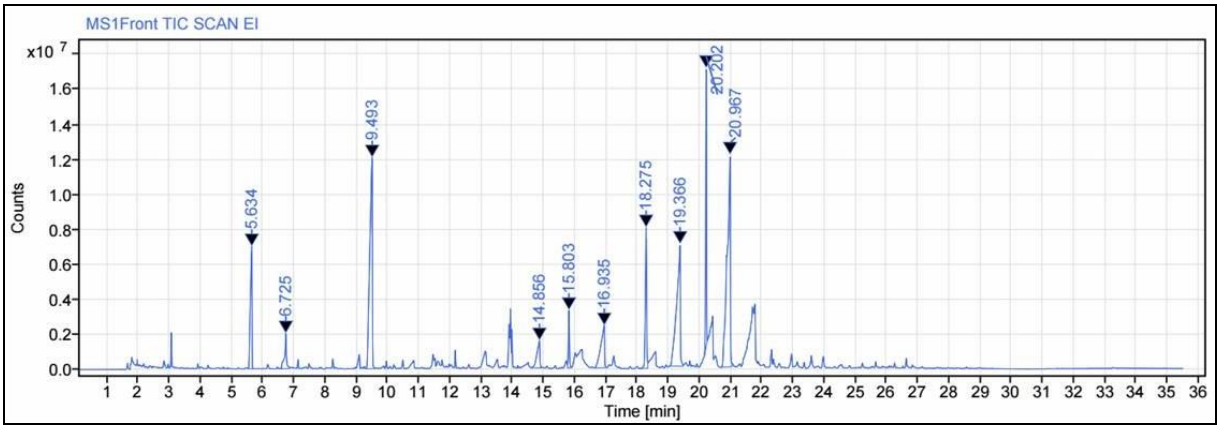

Fig 6: GC MS Chromatogram of KAE

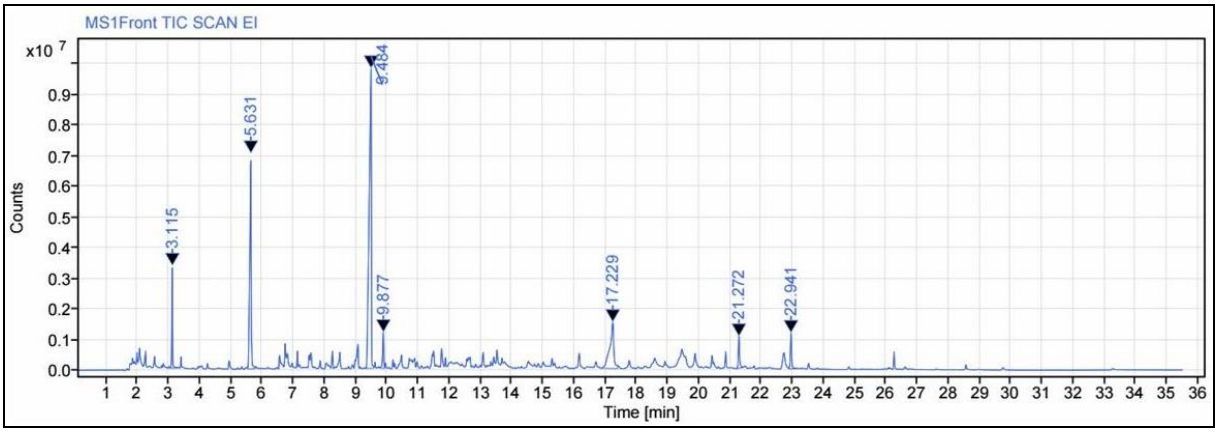

Fig 7: GC MS Chromatogram of SAE

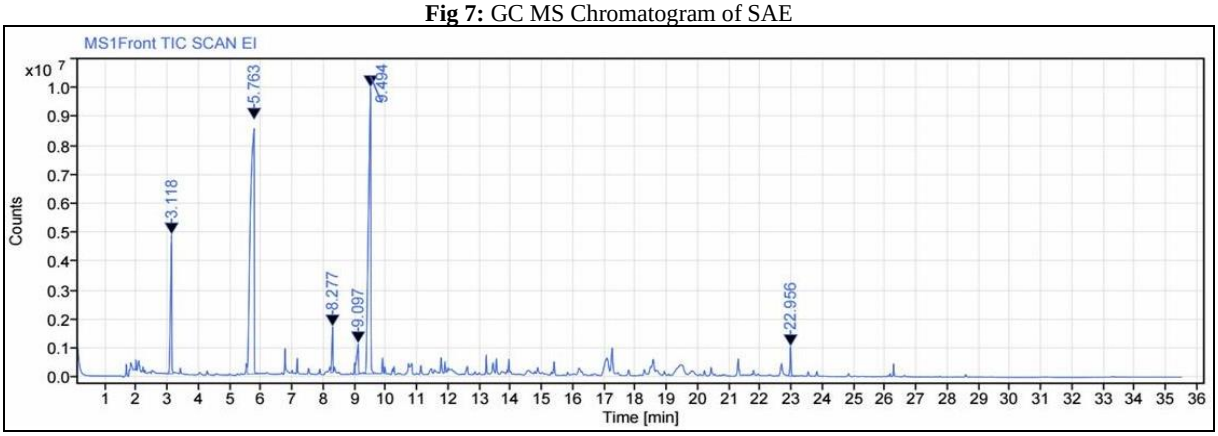

Fig 8: GC MS Chromatogram of MAE

Antimicrobial activity of LAE, KAE, SAE and MAE

LAE, KAE, SAE and MAE antimicrobial property was performed with both gram negative bacteria (*E. coli*) and gram positive bacteria (*S. aureus*). Astonishingly, LAE, KAE, SAE and MAE found to show zone of inhibition against both the bacteria (Fig.09).

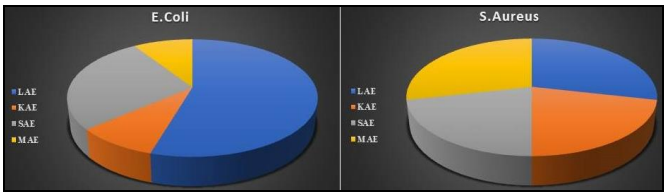

Fig 9: Antimicrobial Property

Moreover, LAE, KAE, SAE and MAE did not hydrolyze RBC suggested its nontoxic property (Fig.10).

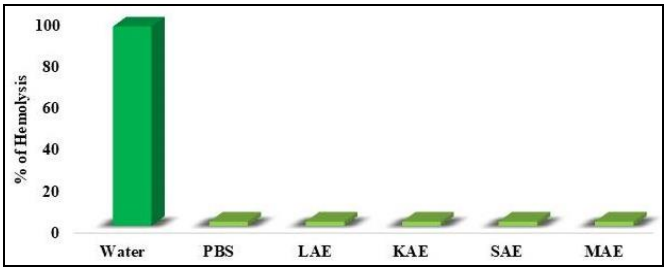

Fig 10: Hemolytic activity

Conclusion

The preliminary characterization of LAE, KAE, SAE and MAE and its antimicrobial activity against both gram positive and gram negative bacteria are presented in the study's conclusion.

Acknowledgments

Authors thank for Mr. Prakash G [CEO of A TO Z ENVIRO TEST HOUSE LLP LAB] to given the opportunity for research work. We are very great full for all research associates of ENVIRO TEST HOUSE LAB namely Prashanth, Kavya, Vathsala, Shridevi, Mounika, Lavanya, Vani and Manjunath for their support during our research work.

Declaration of Conflict of Interest

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

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