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Study the Role of *Guizotia Abyssinica* Secondary Metabolites [GASM] on Gram Negative Bacteria and Human Citrated Plasma

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Abstract*—The present study deals with the extraction, depiction and the role of Guizotia Abyssinica Secondary Metabolites [GASM] on gram negative bacteria and human citrated plasma. Guizotia Abyssinica seeds with holds numerous macro and micro nutrients which include vitamins, proteins, carbohydrates, minerals and mainly oil content. Guizotia Abyssinica oil mainly contains poly unsaturated and saturated fatty acids. Guizotia Abyssinica seeds were subjected to Sox-let-extraction with methanol and final crude was termed as GASM [Guizotia Abyssinica Secondary Metabolites]. GASM was initially screened was its biochemical content and also it was subjected to instrumentation method such as RP-HPLC and GC-MS. Interestingly both GC-MS and RP-HPLC peaks of GASM demonstrate that it should be presence of maximum amount of polyaromatic rings compounds like lipids, alkaloids, flavonoids, steroids and other secondary metabolites. In addition, plasma re-calcification time was performed using Platelet Rich Plasma [PRP] and Platelet Poor Plasma [PPP] for GASM; unfortunately it doesn't show any activity like initiating the clotting process or delaying clotting process in both PRP and PPP. Apart from all this above mentioned assays, fortunately we found that non-toxic property of GASM when we perform non-toxic assay using packed RBC. Surprisingly, GASM exhibit anti-bacterial property against only gram negative bacteria*.

***Keywords*—** *Guizotia Abyssinica Secondary Metabolites (GASM), GC-MS, RP-HPLC, PRP, PPP, Antibacterial property and Non- toxic property*.

I. INTRODUCTION

Guizotia Abyssinica is yellow flowers bearing herbaceous plant and its belongs to Asteraceae family [1]. It is the richest source of edible oil which is largely and mainly cultivated in the Ethiopia's islands due to its suitable nature soil environment for its growth and development [2]. *Guizotia Abyssinica* seeds delivers almost 50-65% of Ethiopia's edible oil needs [3]. Totally there are six kinds of verities of species in the genus of *Guizotia,* where there is more or less five species are native of Ethiopia's islands which is also including Niger [4]. And remaining one species namely *Tanzania* is majorly found to be grown in Nepal, Zimbabwe, Malawi, Uganda and Bangladesh [5]. *Guizotia Abyssinica* withholds high amount of linoleic acid and due to its unsaturated fatty acid nature it has numerous medicinal benefits [6]. The phytochemicals present in the *Guizotia Abyssinica* possess many health benefits such as antioxidant capability which in turn beneficial in the anticancer activity and also it is good for heart diseases [7]. *Guizotia Abyssinica* seeds oil can be also utilized in the soaps, paints and lubricants manufacturing industries. With addition to main course of fatty acids profile of the *Guizotia Abyssinica* seed oil; it contains approximately 1% of arachidonic acid, 3% of linolenic acids, 5% of oleic acids, 8% of palmitic acids and 6% of stearic acids [8]. Inspite of its evolved therapeutic application of *Guizotia Abyssinica* seeds phytoconstituent, there is no much research on secondary metabolites applications. Thus, present study deals with the secondary metabolites preliminary study and its therapeutic applications of *Guizotia Abyssinica*.

II. MATERIALS AND METHODS

All the chemicals used were of analytical grade. Fresh human blood was collected from healthy donors for Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP).

Preparation of GASM

Guizotia Abyssinica seeds were purchased from local market. From the purchased seeds secondary metabolites was extracted by the solvent methanol using Soxhlet extraction method. The finally obtained extract was termed as Guizotia Abyssinica Secondary Metabolites (GASM) and it utilized for further assays.

Preliminary phytochemical screening of GASM

GASM was screened for alkaloids, anthroquinones, flavonoids, phenols, steroids, tannins, terpenoids, [9] cardiac glycosides, saponins, [10] phlobatannin, [11] reducing sugars, [12] volatile oils, [13] carbohydrates and protein/amino acids. [14, 15].

Reverse Phase High Performance Liquid Chromatography analysis of GASM

GASM 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 [16].

GC-MS analysis of GASM

GASM was analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Capture Negative Ion Chemical Ionization (ECNICI)

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mode with capillary column (30m lengthX0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.9%) gas was used as carrier gas at the flow rate of 6ml/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 (5min hold) with an increase in temperature of 10°C/min to 150°C (5min hold), thereafter 20°C/min to 200°C (5min hold), 25°C/min ramp to 250°C (5 min hold), 30°C/min ramp to 280°C (5 min hold). Total run time 41 min. Sample was analyzed in GC-MSD, model 5977B Agilent Make. Mass spectrum was taken at 80ev; a scan interval of 0.5s [17].

Preparation of Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP)

The PRP and PPP were prepared as described by Ardlie and Han [18]. The platelet concentration of PRP was adjusted to 3.1×10^8 platelets/mL with PPP. The PRP has to be used within 2hr from the time of blood drawn at 37°C. All the above preparations were carried out using plastic wares or siliconized glass wares.

Plasma re-calcification time of GASM

The plasma re-calcification time was determined according to the method of Quick [19]. Briefly, the GASM (1-10µL) was pre-incubated with 0.2mL of citrated human plasma in the presence of 10mM Tris HCl (20µL) buffer pH 7.4 for 1min at 37°C. Clotting time was recorded after the addition of 20µL $CaCl_2$ (0.25M) to the pre-incubated mixture.

Direct hemolytic activity of GASM

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 GASM (0-100µL) 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 [20]. 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 GASM

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 agar petri plates were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates evenly with the help of a glass spreader. Wells were made on the solidified agar using a cork borer. The test solution was made by dissolving 10mg of GASM in 1.0mL of water to get 10mg/mL concentration followed by sonication for 2min. The 50µL of this test solution containing 0.5mg of GASM added into the respective wells. The standard antibiotic drug ciprofloxacin 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 [21]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported

III. Results and Discussion

Physical and chemical Characterization of GASM

The extracted GASM was observed pale yellow in color, odorless with lower viscosity. In addition, it was found to present alkaloids, flavonoids, carbohydrates, phenolic compounds and steroids as per preliminary screening of GASM (Table 01).

TABLE 01: Preliminary Phytochemical analysis of GASM

SL No	Phytochemical analysis	Test/Reagents	Result
1	Alkaloids	DragendorfFs test	Positive
2	Anthroquinones	Borntrager's test	Negative
3	Cardiac glycosides	Kellar-Kiliani test	Negative
4	Flavonoids	Shinoda test	Positive
5	Phenols	Phenol test	Positive
6	Reducing sugars	Fehling test	Positive
7	Saponins	Frothing test/Foam test	Negative
8	Steroids	Liebermann-Burchardt test	Positive
9	Tannins	Braemer's test	Negative
10	Terpenoids	Liebermann-Burchard	Negative
11	Carbohydrates	Molish's test	Positive
12	Protein/Amino acids	Biuret test/ Ninhydrin test	Negative

RP-HPLC analysis of GASM

GASM HPLC chromatogram conforms the presence of 5 different types of compounds by eluting 5 peaks at different retention time in reverse phase HPLC attached to Variable Wavelength Detector. Sample was eluted at 295nm at room temperature (Fig.01).

Secondary metabolites were identified in GC–MS analysis of GASM

GASM found to presence of 10 different set of compounds (Fig.02). As per GC-MS analysis and MS library information it was found to be presence of 5-hydroxymethylfurfural (Intermediate compound in the synthesis of levulinic acid), indole alkaloid, vinyl benzene moiety flavonoid, quinic acid (phenolic compound), linoleic acid, oleic acid, stearic acid, phytosterol and β-sitosterol compounds. At the retention time of 11.5, 12.1, 14.4, 17.5, 23.4, 25.2, 26.0, 26.5, 38.5 and 39.3 respectively.

Plasma Recalcification Time analysis of GASM

To evaluate the role of GASM on human citrated plasma, plasma re-calcification time was performed using both human Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP). Unfortunately, GASM doesn't exhibit neither pro-Coagulant nor anticoagulant properties. (Fig.03). Plasma re-calcification time plays pivotal role in identifying the pro-coagulant and anti-coagulant property of said molecule or herbal biomolecules [22]. Anti-coagulants or pro-coagulants are very much useful in the treatment of thrombotic disorders [23]. Therefore, identification of novel pro-coagulants or anti-coagulants from natural products which bear without any side effects found to be sounds good [24]. Several, pro-coagulants and anti-coagulants were isolated from plants sources [25].

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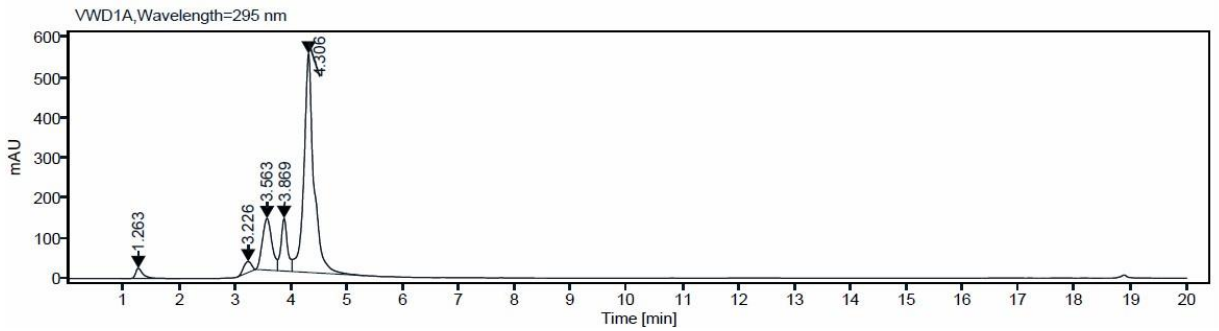

Fig.01 RP-HPLC chromatogram

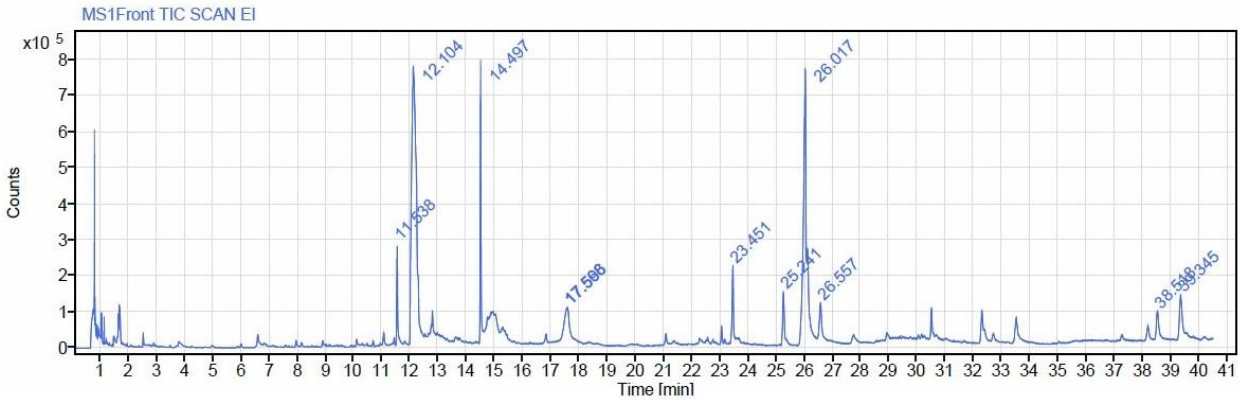

Fig.02 GC-MS Chromatogram of GASM

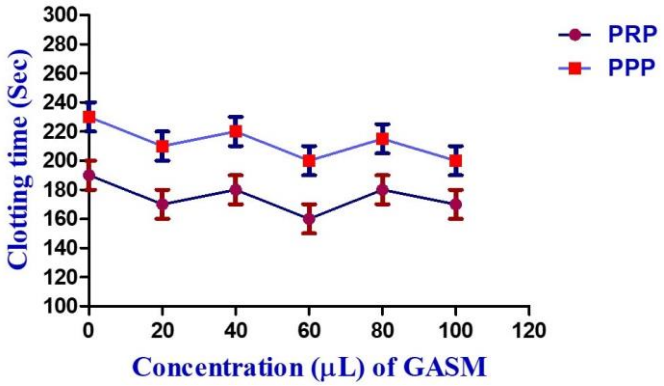

Fig.03 Plasma re-calcification time of GASM

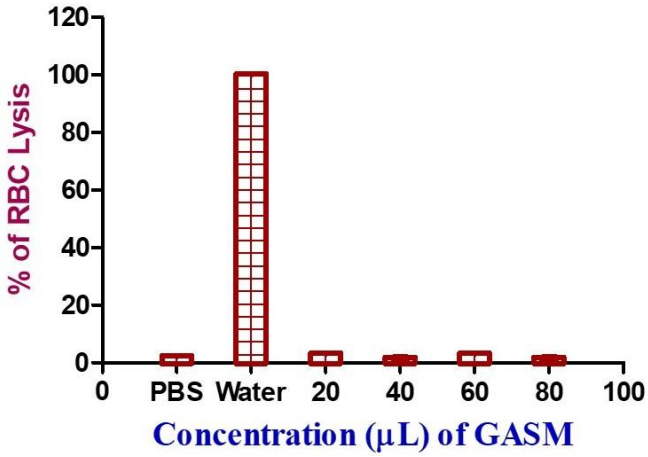

Fig.04 Hemolytic activity of GASM

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

Fig.05 Antibacterial activity of GASM against S. aureus and E. coli. A. Antibacterial activity of GASM against *E.Coli* B. Antibacterial activity against *S.aureus*

Antimicrobial activity of GASM

GASM antimicrobial property was performed with both gram negative bacteria (*E.coli)* and gram positive bacteria (*S.* aureus). Surprisingly, GASM found to show zone of inhibition against only on gram negative bacteria (Fig.05). This result could be due to the different composition of the cell wall between Gram-positive and Gram-negative bacteria.

IV. CONCLUSION

In conclusion, this study demonstrates the preliminary characterization of GASM and its antimicrobial property which is very specific to gram negative bacteria.

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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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