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Study the role of Trigonella foenum-graecum L. Buffer Extract [TFGBE] on Plasma Coagulation cascade

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Abstract
The scope of this study reveals the preliminary screening of Trigonella foenum-graecum L. Buffer Extract [TFGBE] and its role on plasma coagulation cascade. Initially, Trigonella foenum-graecum L. was subjected to sox-let-extraction using phosphate buffer and the extract was termed as Trigonella foenum-graecum L. Buffer Extract [TFGBE]. TFGBE shows the presence of alkaloids, flavonoids, terpenoids, glycosides and etc., in its preliminary screening. GC-MS analysis demonstrate the presence of several micro and macro molecules irrespective of its functional groups and interestingly some of the isomers are also eluted in the GC-MS analysis of TFGBE. Furthermore, TFGBE was subjected for plasma re-calcification time using Platelet Rich Plasma [PRP] and Platelet Poor Plasma [PPP]; interestingly, it exhibit weak anti-coagulant property as it prolongs the clotting time from 180sec to 230sec in PRP and from 210sec to 260sec in PPP and get saturated at the concentration of 60µg. Moreover, TFGBE shows non-toxic property as it was unable to cleave packed RBC in in-vitro study.

Key words: *Trigonella foenum-graecum L. Buffer Extract (TFGBE), GC-MS, PRP, PPP, Anti-coagulant and Non- toxic property.*

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I. Introduction

Trigonella foenum-graecum L. is generally known as Fenugreek which is one of among oldest medicinal or herbal plants [1]. In Greeks and romans they are widely using fenugreek seeds for cattle fodder, so the Latin *Foenum Graecum* means Greek Hay [2]. In contrasting, fenugreek seeds were used for treatment of weakness and edema legs in Chinese medicine [3]. But in India fenugreek seeds were commonly used as a lactation stimulant [4]. Similarly, in Egypt fenugreek were used as fragrance and mainly used to preserve mummies [5]. And also fenugreek seeds were widely cultivated in India and Northern Africa region, fenugreek dried seeds were broadly used as flavoring agent due to its rich source of minerals, vitamins, crude fiber, fat and protein [6]. Due to withhold of enormous phytochemicals in the fenugreek it have immense therapeutical application like aiding digestion, hypoglycemic, anti-hyper-lipidemic, fat absorption, reduce cholesterol, regulation of blood lipid, anti-oxidant, anti-carcinogenic, gastro-protective, regulate hyper-thyroidism, increase milk production, reducing body weight, treatment of arthritis and also possess anti-microbial activities [7]. As fenugreek seeds withhold more ploy-cyclic-compounds such as flavonoids, alkaloids and saponins it is widely used throughout worldwide as an anti-inflammatory agent [8]. Thus, fenugreek seeds were majorly used as herbal medicine through food. Cardiovascular disorder including thrombosis, ischemic heart disease, stroke and coronary heart disease are the main leading cause for drastic increase in the immortality rate throughout the world wide [9]. Formation of thrombus due to the disruption of homeostasis can cause vascular blockage, myocardial, athero-thrombotic and cerebral necrosis which finally leads to death [10]. Blood coagulation factors and platelets play a pivotal role in the formation of blood clot, so regulation of coagulation cascade is major task for researchers to induce or reduce blood clots [11]. Thus, identification of novel anti-coagulant and pro-coagulant agents from herbal source acts a major role in the regulation of plasma coagulation cascade [12].

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 TFGBE

Trigonella foenum-graecum L. seeds were purchased from local market. It was subjected for Soxhlet extraction method using phosphate buffer to obtain the buffer extract. The finally obtained extract was termed as *Trigonella foenum-graecum L.* Buffer Extract (TFGBE) and it utilized for further assays.

Preliminary phytochemical screening of TFGBE

TFGBE was screened for terpenoids, phytosterol, tannin, phenolic, glycoside, saponins, flavonoids, carbohydrates, proteins, steroids and lipids [13].

GC-MS analysis of TFGBE

TFGBE 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 (4min hold), 30°C/min ramp to 280°C (4min hold). Total run time 35.5min. Sample was analyzed in GC-MSD, model 5977B Agilent Make. Mass spectrum was taken at 70ev; a scan interval of 2.92s [14].

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

The PRP and PPP were prepared as described by Ardlie and Han [15]. 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 TFGBE

The plasma re-calcification time was determined according to the method of Quick [16]. Briefly, the TFGBE (10-70µg) 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 TFGBE

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 TFGBE (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 [17]. 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.

III. Results and Discussion

Chemical Characterization of TFGBE

The TFGBE was found to be withholding of terpenoids, phytosterol, phenolic-compounds, glycosides, saponins, flavonoids, carbohydrates, alkaloids, steroids and lipids as per the preliminary screening (Table 01).

SL NO	Phytochemical Analysis	Results
01	Terpenoid	Present
02	Phytosterol	Present
03	Tannin	Absent
04	Phenolic	Present
05	Glycoside	Present
06	Saponin	Present
07	Flavonoid	Present
08	Carbohydrates	Present
09	Proteins	Absent
10	Alkaloid	Present
11	Steroids	Present
12	Lipids	Present

Table: 01

Phytochemical analysis of TFGBE in GC–MS

TFGBE found to presence of 11 different set of compounds, as per GC-MS analysis it found to elute 11 peaks at the retention time of 1.7, 2.6, 2.9, 3.9, 18.6, 19.5, 23.4, 24.7, 26.2, 28.1 and 28.5 respectively (Fig.01).

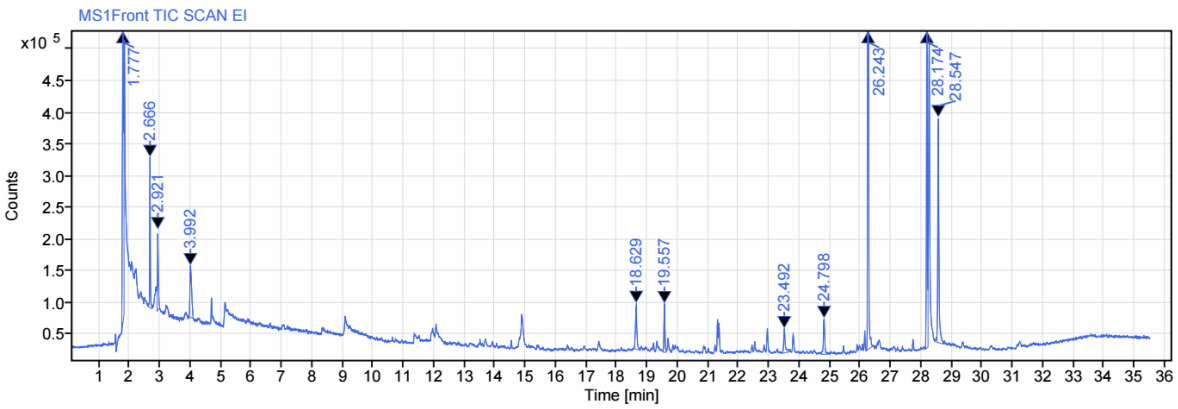

Fig: 01

Plasma Recalcification Time analysis of TFGBE

In order to study the role of TFGBE on human citrated plasma, plasma-re-calcification time was done using Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP). Nevertheless, TFGBE exhibit anti-coagulant property but it is increasing the clotting time in seconds only that is only 50sec from the control time in both PRP and PPP. And also it is reaching saturation level at the concentration of 60µg of TFGBE. In PRP it is increasing the clotting time from 180sec to 230sec and in PPP it is increasing the clotting time from 210sec to 260sec. Thus, it is concluded that TFGBE exhibiting weak anticoagulant property in both PRP and PPP (Fig.02). Identifying the novel anti-coagulant and pro-coagulant agents from herbal seeds is a major task for all researchers in-order to treat thrombotic disorders without any side effects [18]. Even though at present we have synthetic anti-coagulants and pro-coagulants, in-order to minimize the side effects causing from the synthetic drugs, herbal medicines novel exploration in the field of cardiovascular disorders is very mush essential for present lifestyle [19]. As initial step in the formation of thrombus is none other than the formation of platelet plaque at the site of injury, eventually plasma coagulation factors get activated and help in the formation of thrombus [20]. Hence, regulation of plasma coagulation cascade is very much important which is occur in the inside the blood vessel. Anti-coagulants help in the treatment of thrombotic disorders and pro-coagulants helps in the treatment of hemophilia disorders [21].

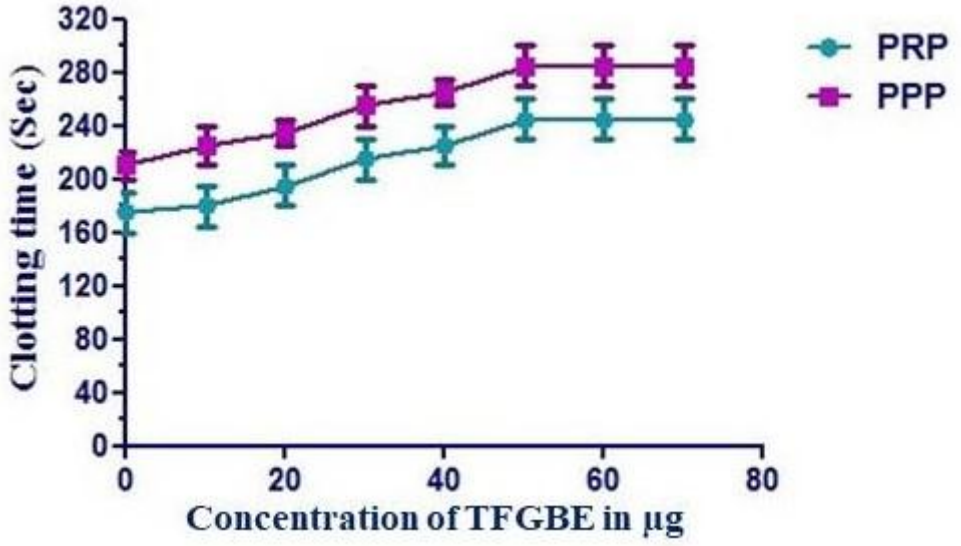

Fig: 02

Furthermore, TFGBE did not hydrolyze RBC suggested its nontoxic property (Fig.03).

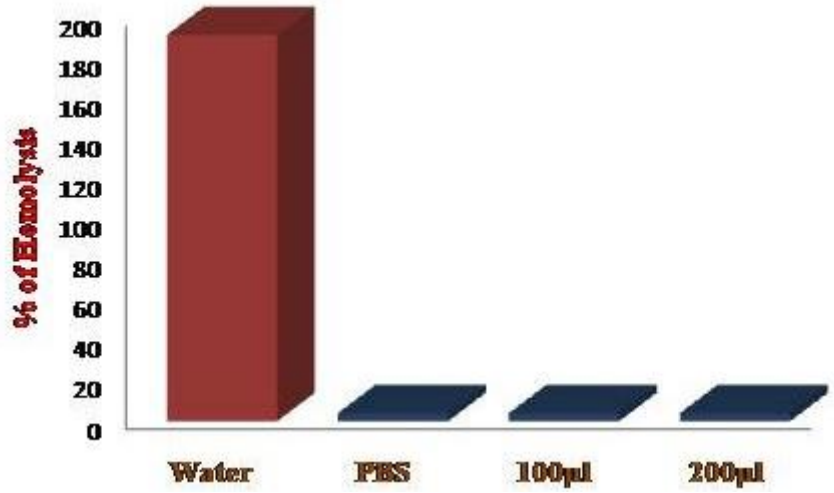

Fig: 03

IV. Conclusion

In conclusion, this study demonstrates the preliminary characterization of TFGBE and its weak anti-anticoagulant property in both PRP and PPP.

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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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