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Seamless study of Coriandrum Sativum Buffer Extract [CSBE] on its therapeutical applications

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Abstract:

The existent study pacts with the Coriandrum Sativum Buffer Extract [CSBE] extraction, preliminary screening, depiction and the role of CSBE on microbial cultures, packed RBC and human plasma. Coriandrum Sativum seeds were subjected to Soxhlet extraction using phosphate buffer and the final obtained extract was named as Coriandrum Sativum Buffer Extract [CSBE]. CSBE shows the withholding of terpenoids, glycosides, saponins, flavonoids, carbohydrates, steroids and lipids when it subjected for preliminary screening. CSBE proves the presence of many micro and macro molecules in the extract, as it elute 6 peaks and 10 peaks when we subjected for HPLC and GC-MS respectively. Unfortunately, CSBE does not show any role on microbial cultures as it unable to exhibit zone of inhibition when it incubated with E.coli and S.aureus. Furthermore, CSBE does not show any activity when it treated with Platelet Rich Plasma [PRP] and Platelet Poor Plasma [PPP] during the analysis plasma re-calcification time assay. But, CSBE demonstrate anti-platelet activity for ADP receptor as it inhibits the platelet aggregation by 68% at the concentration of 300µg. In addition, CSBE exhibit non-toxic property as it unable to rupture the membrane of packed RBC.

Keywords — **Coriandrum Sativum Buffer Extract [CSBE], GC-MS, RP-HPLC, PRP, PPP, Antiplatelet and Non- toxic property.**

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I. INTRODUCTION

Coriandrum Sativum commonly termed as Coriander which belongs to the Apiaceae family. Basically, Coriandrum Sativum is originated from the Mediterranean region [1]. But today it is widely cultivated in North Africa, Europe and Asian countries. Coriandrum Sativum is herbaceous plant that grows ranging from 25 to 60cm in height [2]. Coriandrum Sativum will be used both as a spice and in the treatment of digestive disorders including indigestion, nausea and dysentery and so on, while the coriander leaves on the other hand can help in stimulating appetite and easy digestion [3]. Coriandrum Sativum reported several pharmacological effects which includes antioxidant activity, antifungal activity, antiviral activity, anticoagulant activity, anti-helminthic activity, sedative-hypnotic activity, anticonvulsant activity, diuretic activity, a cholesterol lowering activity, protective effects against lead toxicity, antifungal property, anticancer activity, anxiolytic activity or hepato-protective activity, anti-protozoal activity, anti-ulcer activity, post-coital anti-fertility activity and also heavy metal detoxification properties [4]. Coriandrum Sativum seeds are generally used as a flavouring agent for many foods such as fast foods, home-made foods, restaurant foods, fish and meats, bakery and confectionery products [5]. Coriandrum Sativum majorly impact on human health as

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common anti-oxidants due to the presence of active flavonoids include quercetin, keampferol, rhamnetin and apigenin, these compounds are known to inhibit free radicals produced in the cellular system, when they are obtained through the diet [6]. Due to the more valuable therapeutical application of Coriandrum Sativum it is widely used as one of the herbal medicine in recent days of modern era [7]. Thus, this current study focuses on Coriandrum Sativum Buffer Extract [CSBE] on its vital role on plasma coagulation cascade and plasma platelets.

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 CSBE

Coriandrum Sativum seeds were purchased from local market. It was subjected for Soxhlet extraction apparatus using PBS buffer. The finally obtained extract was termed as Coriandrum Sativum Buffer Extract [CSBE] and it utilized for further assays.

Preliminary phytochemical screening of CSBE

CSBE was screened for terpenoids, phytosterol, tannin, phenolic, glycoside, saponins, flavonoids, carbohydrates, proteins, steroids and lipids [8-12].

Reverse Phase High Performance Liquid Chromatography analysis of CSBE

CSBE was subjected to RP-HPLC using C18 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 [13].

GC-MS analysis of CSBE

CSBE was analysed 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 3.1×108 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 CSBE

The plasma re-calcification time was determined according to the method of Quick [16]. Briefly, the CSBE (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 CaCl2 (0.25M) to the pre-incubated mixture.

Platelet aggregation assay of CSBE

The turbid metric method of Born was followed using a chronology dual channel whole blood/optical Lumi aggregation system (Model-700). Aliquots of PRP were pre-incubated with various concentrations of CSBE (0-300µg) in 0.25mL reaction volume. The platelet aggregation was initiated independently by the addition of ADP agonist and followed for 6min [17].

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Direct haemolytic activity of CSBE

Direct haemolytic 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 CSBE (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 [18]. 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 CSBE

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 50mg of CSBE in 1.0mL of methanol to get 50mg/mL concentration followed by sonication for 2min. The 100µL of this test solution containing 5mg of CSBE 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 [19]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported.

ICP-OES analysis of CSBE

CSBE 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 12RPM pump speed, 25sec sample uptake time, 30sec of rinse time, 5sec, read time, 1.2KW RF power, 15sec stabilization time, Axial viewing mode, 8mm viewing height, 0.7L/Min nebulizer flow, 12L/Min plasma flow, 0.75L/Min Aux flow.

III. RESULTS AND DISCUSSION

Chemical Characterization of CSBE

CSBE was found to be showing the presence of terpenoids, glycosides, saponins, flavonoids, carbohydrates, steroids and lipids as per the preliminary screening of CSBE (Table 01). It also demonstrate that it possess several minerals like Aluminium, iron, manganese, barium, boron, molybdenum, nickel and zinc (Table 02).

SL NO.	Phytochemical Analysis	Result
01	Terpenoid	Present
02	Phytosterol	Absent
03	Tannin	Absent
04	Phenolic Compound	Absent
05	Glycosides	Present
06	Saponin	Present
07	Flavinoids	Present
08	Carbohydrates	Present
09	Proteins	Absent
10	Steriods	Present
11	Lipids	Present

Table 01

SL No.	Name Of The Metal	CSBE in ppm
01	Aluminum	0.55
02	Boron	0.24
03	Barium	0.21
04	Cadmium	0.00
05	Copper	0.10
06	Iron	0.74
07	Manganese	0.62
08	Molybdenum	0.01
09	Nickel	0.02
10	Lead	0.01
11	Zinc	0.61

Table 02

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RP-HPLC analysis of CSBE

CSBE shows 6 different types of compounds by eluting 6 peaks at different retention time in reverse phase HPLC attached to Variable Wavelength Detector. Sample was eluted at 216nm at room temperature (Fig.01).

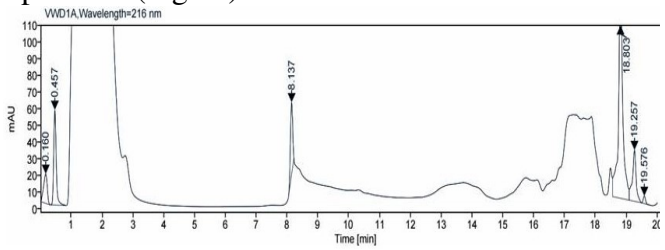

Fig.01 HPLC CHRMATOGRAM OF CSBE

GC–MS analysis of CSBE

CSBE found to shown 10 different set of compounds (Fig.02). In GC-MS analysis it was found to elute those 10 major peaks at the retention time of 1.8, 2.7, 7.8, 9.4, 11.2, 13.1, 15.2, 26.6, 28.2 and 28.9 respectively.

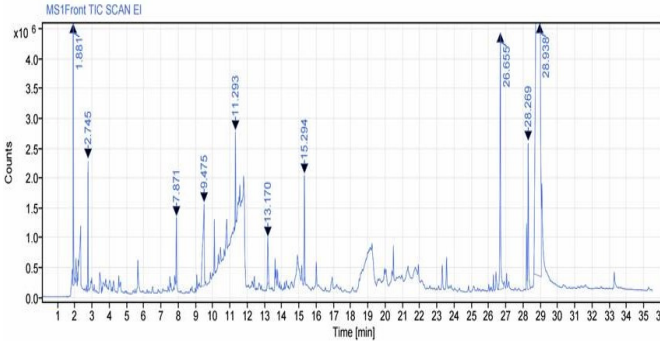

Fig.02 GC-MS CHRMATOGRAM OF CSBE

Antiplatelet activity of CSBE

To access the role of CSBE on plasma platelets, platelet aggregation assay was performed by using ADP as an agonist in Platelet Rich Plasma [PRP]. Astonishingly, CSBE inhibited the ADP induced platelet aggregation, around 68% of inhibition was found to shown by the CSBE at the concentration of 300µg in in-vitro study (Fig.03). It is well established in blood research that activated platelets equally contribute along with fibrin clot formed by the coagulation factors through cell signalling coagulation cascade to arrest the bleeding at the site of injury [20]. So, inhibiting or enhancing the activated platelets play a pivotal role in the hyper coagulation and haemophilia disorders [21].

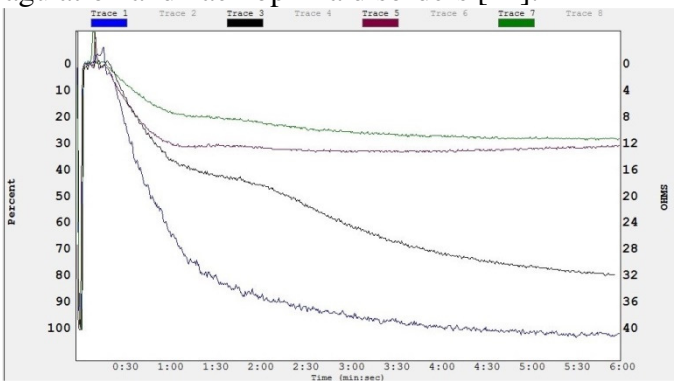

Fig.03 ANTIPLATELET ACTIVITY OF CSBE

Moreover, CSBE did not show any role on plasma re-calcification time when it treated with PRP and PPP, which intern suggests there is no role either anti or pro coagulant property of CSBE. Furthermore, CSBE fail to create zone of inhibition in both E.coli and S.aureus culture medias, which suggests that there is no role of CSBE on pathogenic cultures. In addition CSBE unable to cleave packed RBC suggests its nontoxic property (Fig.04).

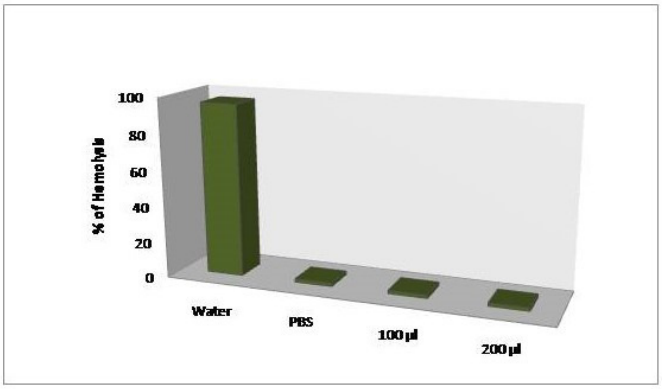

Fig.04 HAEMOLYTIC ACTIVITY OF CSBE

IV. CONCLUSIONS

In conclusion, this study reveals the preliminary characterization, HPLC and GC-MS analysis of CSBE and its anti-platelet property.

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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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