E-ISSN: 2583-9667
Indexed Journal
Peer Reviewed Journal
https://multiresearchjournal.theviews.in

Received: 19-07-2024
Accepted: 23-08-2024

INTERNATIONAL JOURNAL OF ADVANCE RESEARCH IN MULTIDISCIPLINARY

Volume 2; Issue 3; 2024; Page No. 337-341

Biomedical applications of *Phyllanthus emblica* Buffer Extract [PEBE] and *Citrus reticulata,* Rutaceae Buffer Extract [CRRBE]

[1]Nandish GA, [2]Meghana S, [3]Likhitha G, [4]Sangita Roy, [5]Evawe Prabha, [6]Deepa G, [7]Vani Krishna Naik and [8]Sharath Kumar MN

[1-5]Department of Studies and Research in Biochemistry, The Oxford College of Science Bangalore, Karnataka, India
[6, 7]A TO Z Enviro Test House LLP, Electronic City Phase-2, Bangalore, Karnataka, India
[8]Senior Scientist, A TO Z Enviro Test House LLP, Electronic City Phase-2, Bangalore, Karnataka, India

DOI: https://doi.org/10.5281/zenodo.13365408

Corresponding Author: Sharath Kumar MN

Abstract

Phyllanthus emblica Buffer Extract [PEBE] and *Citrus reticulata,* Rutaceae Buffer Extract [CRRBE] characterization and its role on biomedical application are the scope of this study. In preliminary characterization PEBE shows the presence of flavonoids, alkaloids, proteins and phenolic compounds. In the other hand CRRBE shows the presence of terpenoids, saponins, proteins, alkaloids and phenolic compounds. To verify the presence of phytoconstituent, both PEBE and CRRBE were subjected to HPLC and GC-MS analysis. Interestingly, PEBE elutes 5 and 7 peaks in HPLC and GC-MS respectively. Whereas, CRRBE elutes 3 and 9 peaks in HPLC and GC-MS respectively. In addition, both PEBE and CRRBE found to be presence of several minerals such as aluminium, boron, copper, iron and etc. Furthermore, PEBE and CRRBE exhibit anti-microbial activity by inhibiting the growth of pathogenic organisms in respective media. Moreover, both PEBE and CRRBE exhibit non-toxic property as it is unable to cleave packed RBC.

Keywords: *Phyllanthus emblica* Buffer Extract [PEBE], *Citrus reticulata,* Rutaceae Buffer Extract [CRRBE], GC-MS, RP-HPLC, Antibacterial property and Non- toxic property

Introduction

In India, *Phyllanthus emblica* is referred to as amla or Indian gooseberry [1]. Higher concentrations of polyphenols such as gallic and ellagic acid, as well as other tanins, minerals, vitamins, amino acids, fixed oils, and flavonoids such as rutin and quercetin, are among the phytoconstituent of Amla [2]. "Rejuvenating herb" is how amla is utilized in the traditional Indian medical system [3]. It contains a variety of phenolic compounds and is a strong nutritional source of minerals, amino acids, and vitamin C [4]. It is also known that amla extract has strong antioxidant qualities [5]. Amla extract also shields human dermal fibroblasts from oxidative damage [6]. It also has anti-apoptotic and anti-inflammatory properties [7]. Because of its many health and nutritional advantages, amla has become extremely important in Indigenous traditional medical systems, such as Ayurvedic medicine [8]. It is used to treat a number of illnesses, including the common cold, fever, cough, bronchitis, asthma, diabetes, Ophthalmopathy, peptic ulcer, dyspepsia, hyperacidity, leprosy, heart condition, and early onset of grey hair [9]. Citrus aurantium, sometimes referred to as bitter orange, is a fruit that ripens to reveal a high concentration of several phytoconstituents that vary according on the portion of the fruit [10]. Orange peel, or *Citrus reticulata*, Rutaceae, is a rich source of minerals, flavonoids, an alkaloid called p-synephrin, and other secondary metabolites that are beneficial to health [11]. The primary phytochemical components include tannin and naringin content, ferric reducing antioxidant power (FRAP), DPPH free radical scavenging activity, total flavonoid

content, total phenolic content, and antioxidant capacity [12]. The highest concentration of total phenolic and flavonoid content was found in the fruit membrane [13]. Their biological qualities include antibacterial, anticancer, anti-obesity, antioxidant, pesticidal, and antidiabetic properties, among other medicinal potentials [14].

Materials and Methods

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

Preparation of PEBE and CRRBE

Phyllanthus emblica and *Citrus reticulata*, Rutaceae were purchased from local market and it was subjected to Soxhlet extraction method. The finally obtained extracts were termed as *Phyllanthus emblica* Buffer Extract [PEBE] and *Citrus reticulata*, Rutaceae Buffer Extract [CRRBE] and it utilized for further assays.

Preliminary phytochemical screening of PEBE and CRRBE

PEBE and CRRBE were screened for terpenoids, phytosterol, tannin, phenolic, glycoside, Saponin, flavonoid, carbohydrates, proteins, steroids and alkaloids [15].

Reverse phase high performance liquid chromatography analysis of PEBE and CRRBE

PEBE and CRRBE 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 [16].

GC-MS analysis of PEBE and CRRBE

PEBE and CRRBE 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 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. Sample was analyzed in GC-MSD, model 5977B Agilent Make. Mass spectrum was taken at 70ev; a scan interval of 2.92s [17].

Direct hemolytic activity of PEBE and CRRBE

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 PEBE and CRRBE (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 PEBE and CRRBE

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

ICP-OES analysis of PEBE and CRRBE

PEBE and CRRBE 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 [20].

Results and Discussion

Characterization of PEBE and CRRBE

PEBE and CRRBE were found to presence of phenolic compounds, flavonoids, proteins, alkaloids and terpenoids, phenolic compound, saponins, proteins, alkaloids respectively (Table 01). PEBE and CRRBE shows the presence of several minerals such as aluminium, boron, barium, copper, iron, manganese, lead, zinc and etc., (Table 02).

Table 1: Show the phytochemical analysis PEBE and CRRBE

SL. No.	Phytochemical Analysis	PEBE	CRRBE
01	Terpenoid	Absent	Present
02	Phytosterol	Absent	Absent
03	Tannin	Absent	Absent
04	Phenolic	Present	Present
05	Glycoside	Absent	Absent
06	Saponin	Absent	Present
07	Flavonoid	Present	Absent
08	Carbohydrates	Absent	Absent
09	Proteins	Present	Present
10	Alkaloid	Present	Present
11	Steroids	Absent	Absent

Table 2: Name of The Metal PEBE and CRRBE In ppm

SL. NO.	Name of The Metal	PEBE In ppm	CRRBE In ppm
01	Aluminium	1.97	249.17
02	Boron	0.09	0.58
03	Barium	0.06	0.50
04	Cadmium	0.00	0.01
05	Copper	0.03	0.18
06	Iron	2.22	169.72
07	Manganese	0.15	4.75
08	Molybdenum	0.01	0.02
09	Nickel	0.02	0.27
10	Lead	0.02	0.09
11	Zinc	0.12	1.35

RP-HPLC analysis of PEBE and CRRBE

PEBE and CRRBE HPLC chromatogram elutes 5 peaks (Fig.01) & 3 peaks (Fig.02) respectively at different retention time in reverse phase HPLC attached to Variable Wavelength Detector. Sample was eluted at 295nm at room temperature.

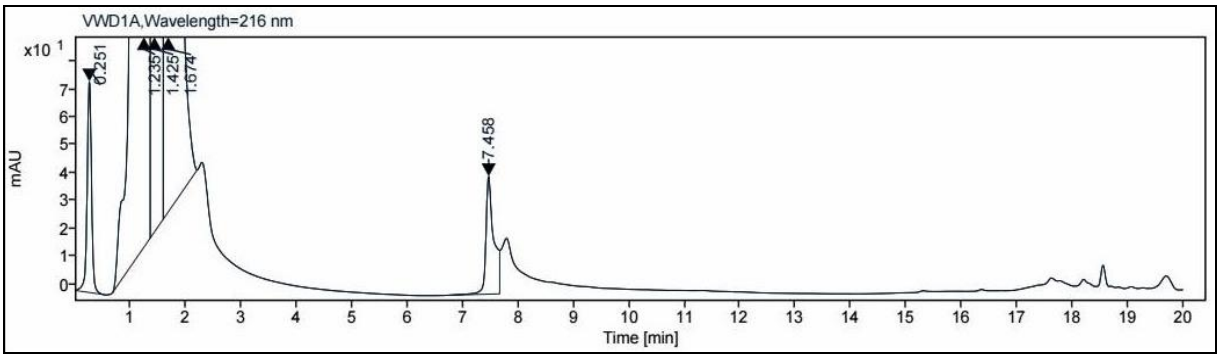

Fig 1: HPLC Chromatogram of PEBE

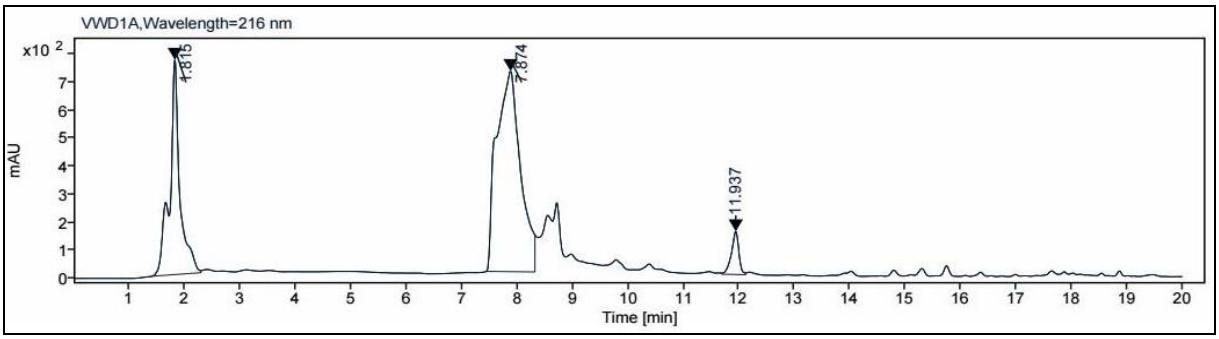

Fig 2: HPLC Chromatogram of CRRBE

GC–MS analysis of PEBE and CRRBE

PEBE and CRRBE GC-MS chromatogram elutes 7 peaks (Fig.03) and 9 peaks (Fig.04) at the retention time of 1.7, 2.1, 2.8, 3.4, 7.8, 16.9, 20.8 and 1.7, 2.8, 5.1, 9.0, 15.2, 20.8, 24.7, 26.2, 28.5 respectively.

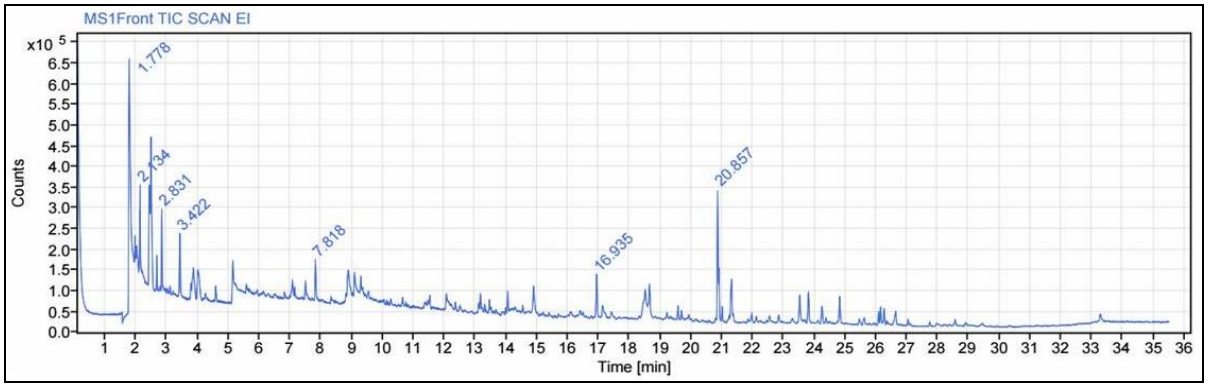

Fig 3: GC MS Chromatogram of PEBE

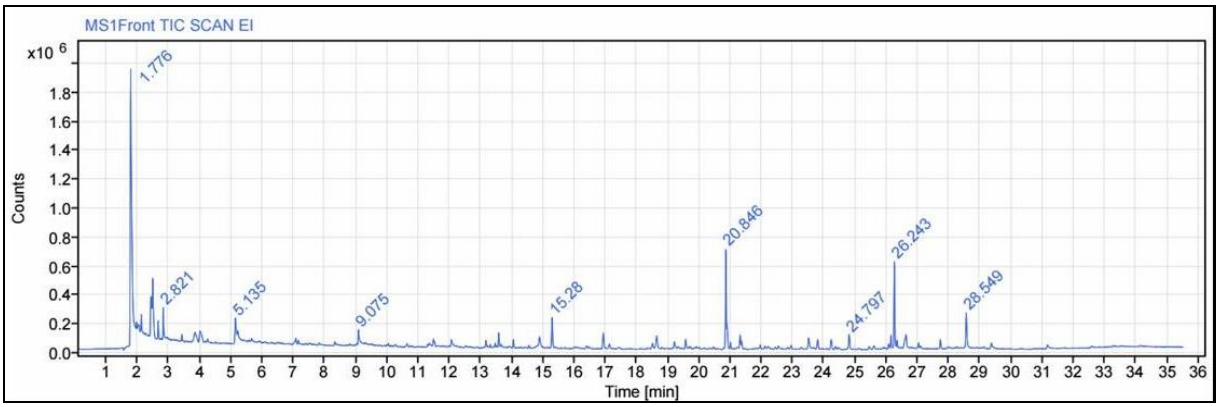

Fig 4: GC MC Chromatogram of CRRBE

Antimicrobial activity of PEBE and CRRBE

PEBE and CRRBE antimicrobial property were performed with both gram negative bacteria (*E. coli*) and gram positive bacteria (*S. aureus*). Surprisingly, both PEBE and CRRBE found to show zone of inhibition against both the bacteria (Fig.05).

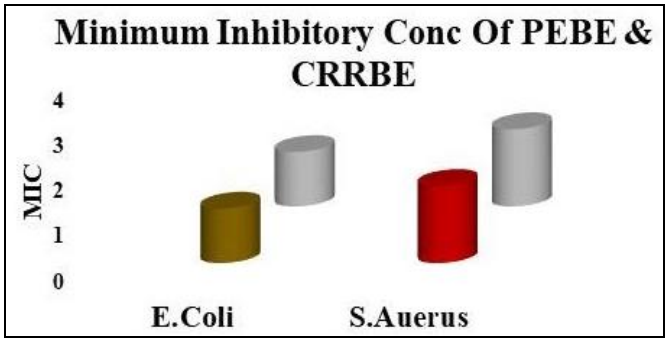

Fig 5: Antimicrobial Property of PEBE & CRRBE

Moreover, PEBE and CRRBE were exhibits non-toxic property as it unable to cleave packed RBC (Fig.06).

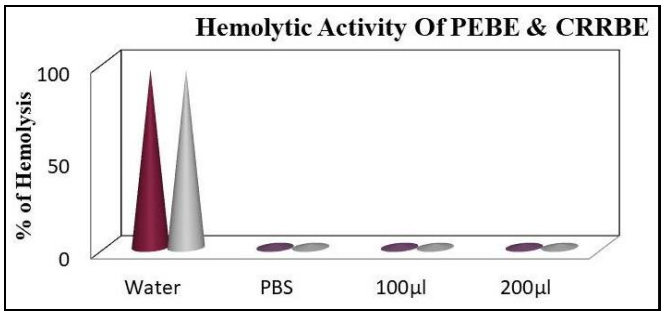

Fig 6: Haemolytic Activity of PEBE & CRRBE

Conclusion

In conclusion, this study demonstrates the preliminary characterization of PEBE and CRRBE and its antimicrobial property against both gram negative and gram positive bacteria.

Acknowledgments

Authors thank for Mr. Prakash [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 Mahamad, Kavya, Vathsala, Shridevi, Mounika, Lavanya, Prashanth 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.

References

1. Zhang LZ, Zhao WH, Guo YJ, Tu GZ, Lin S, *et al*. Studies on chemical constituents in fruits of Tibetan medicine *Phyllanthus emblica*. Zhongguo Zhongyao Zazhi. 2003;28:940-943.
2. Jain PK, Das D, Pandey N, Jain P. Traditional Indian herb Emblica officinalis & its medicinal importance. Innov. J. Ayruvedic Sci. 2016;4:1-15.
3. Liu X, Cui C, Zhao M, Wang J, Luo W, *et al*. Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. Food Chem. 2008;109:909-915.
4. Luanpitpong S, Nimmannit U, Pongrakhananon V, Chanvorachote P. Emblica (*Phyllanthus emblica* Linn.) fruit extract promotes proliferation in dermal papilla cells of human hair follicle. Res. J Med. Plants. 2011;5:95-100.
5. Department of Medical Sciences. Thai Herbal Pharmacopoeia 1. Ministry of Public Health, Nonthaburi, Thailand; c2020. p. 362-369.
6. Teeranachaideekul V, Wongrakpanich A, Leanpolchareanchai J, Thirapanmethee K, Sirichaovanichkarn C. Characterization, biological activities and safety evaluation of different varieties of Thai pigmented rice extracts for cosmetic applications. Pharm. Sci. Asia. 2018;45:140-153.
7. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary Altern. Med. 2012;12:1-12.
8. Rahim NAB, Zakaria N, Dzulkarnain SMH, Azahar NMZM, Abdulla MA. Antioxidant activity of alstonia Angustifolia ethanolic leaf extract. AIP Conference Proceedings. 2017;1891:020012.
9. Hatami T, Emami SA, Miraghaee SS, Mojarrab M. Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of *Artemisia biennis* Willd. Iran. J Pharm. Sci. 2014;13:551-559.

10. Mannucci C, Calapai F, Cardia L, Inferrera G, D'Arena G, Di Pietro M, *et al*. Clinical pharmacology of Citrus aurantium and Citrus sinensis for the treatment of anxiety. Evid. Based Complement. Altern. Med; c2018, p. 3624094.
11. Suntar I, Khan H, Patel S, Celano R, Rastrelli L. An overview on *Citrus aurantium* L.: Its functions as food ingredient and therapeutic agent. Oxid. Med. Cell. Longev; c2018. p. 7864269.
12. Ersus S, Cam M. Determination of organic acids, total phenolic content, and antioxidant capacity of sour *Citrus aurantium* fruits. Chem. Nat. Compd. 2007;43:607-609.
13. Jabri Karoui I, Marzouk B. Characterization of bioactive compounds in Tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activities. BioMed Res. Int; c2013. p. 345415.
14. Sarrou E, Chatzopoulou P, Dimassi-Theriou K, Therios I. Volatile constituents and antioxidant activity of peel, flowers and leaf oils of *Citrus aurantium* L. growing in Greece. Molecules. 2013;18:10639-10647.
15. Harborne. Phytochemical Methods. 2nd ed., New York, London: Springer, Chapman and Hall; c1984. p. 288.
16. Kumar S, Kengaiah J, Ramachandru C, Ashwini C, Sannaningaiah D. Isolated linseed oil from *Linum Usitatissimum* exhibited pro-coagulant property. International Journal of Pharmacy and Biological Sciences. 2019;9(1):1086-1092.
17. Justesen U, Knuthsen P, Leth T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. Journal of Chromatography A. 1998;799:101-110.
18. Sowmyashree G, Bhagyalakshmi M, Girish KS, Kemparaju K, Rangaiah SM, Jane HP. Anticoagulant and Antiplatelet activities of Jackfruit seed extract. I J of Phar and Pharmaceutical sciences. 2015;7(1):187-191.
19. Marulasiddeshwara MB, Dakshayani SS, Sharath Kumar MN, Chethana R, Raghavendra Kumar P, Devaraja S. Facile-one pot-green synthesis, antibacterial, antifungal, antioxidant and antiplatelet activities of lignin capped silver nanoparticles: A promising therapeutic agent. Materials Science & Engineering C; c2017.
20. Shankar, Gowthami, Layavani, Roya S, Prabha E, Ashwini S, *et al*. Identify the role of *Trachyspermum ammi* L. and Helianthus annuus phytoconstituent on microbial cultures. International Journal of Advance Research in Multidisciplinary. 2024;2(3):188-192.