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Therapeutical Application of *Persea American* Phytoconstituent

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Abstract

The extant study pacts with the role of *Persea Americana* methanol and water extracts on microbial cultures. *Persea Americana* Methanol Extract (PAME) and *Persea Americana* water Extract (PAWE) were found to contain terpenoids, Glycosides, flavonoids, carbohydrates and steroids. But only PAME withholds polyphenol compounds, and PAWE contains only saponins, respectively in the extracts when it is subjected to preliminary phytoconstituent characterisation. Surprisingly, both PAME and PAWE confirm more than 10 peaks in analytical assays performed with GC-MS and HPLC instrumentation techniques. Which intern states that more phytochemical compounds are extracted in the extraction of PAME and PAWE. Importantly, PAME and PAWE contain several minerals like Aluminium, cadmium, zinc, barium, etc. Moreover, PAME and PAWE exhibit anti-microbial property by inhibiting the microbial culture growth, such as E. coli, S. aureus, pseudomonas, salmonella and Shigella species. Furthermore, PAME and PAWE were found to be non-toxic in nature as it unable to break RBC cells when we analyse an in-vitro study by using packed RBC.

Keywords: PAME, PAWE, E. *coli*, S. *Aureua*, Pseudomonas, Salmonella, Shigella, Anti-microbial property, Minimum Inhibition Concentration, ICP-OES

1. Introduction

One of the most popular avocado pear cultivars grown in tropical and subtropical regions is the fruit of Persea americana, a member of the Lauraceae family [1], which is consumed all over the world. Different plant sections have been the subject of investigation in recent years. It has been demonstrated that the fruit in particular offers a number of therapeutic benefits [2]. Up to 33% of the edible fruit pulp is oil that is high in monounsaturated fatty acids, which are thought to alter the amount of fatty acids in the heart and kidney membranes and improve the absorption of lutein and α/ß carotene [3]. According to reports, the carotenoid concentration significantly lowers the risk of cancer. Hepatoprotection and wound healing are two further qualities of the oil. Carotenoids, vitamin E, and the polyphenol vitamin C are substances having antioxidant properties that aid in shielding cells from damage caused by free radicals [4].

Persin has been noted to be labile under acid conditions, where it produces a furan ring-containing analog [5]. The leaves of 17 avocado cultivars were investigated for their content of persin, and all but 2 of these contained discernible amounts of this compound (range 0.4-4.5 mg/g) [6]. The glycosylated abscisic acid derivatives (1S,6R)-8-hydroxy abscisic acid-D-glucoside and (1R,3R,5R,8S)-pi-dihydrophaseic acid-D-glucoside were isolated from the seeds of P. americana, although no biological activities were attributed to these compounds. Compounds 20-30 are furanoid constituents of avocados, and have been isolated and structurally characterized or chemically synthesized by several different groups [7]. These compounds have been termed “avocadofurans” and subjected to literature review, primarily from the point of view of the effects of structural modification on their resultant antibacterial, antifungal, and insecticidal activities [8].

Several flavonoids have also been isolated from the leaves and seeds of avocados, with most of these being common flavones of wide distribution in the plant kingdom [9]. Some of these are biologically active, such as quercetin, which showed virustatic effects by inhibiting HIV syncytium formation and viral p24 antigen formation. An extract of avocado leaves inhibited herpes simplex virus type 1 and

Aujeszky's disease virus (ADV), and adenovirus type 3 (AD3) [10]. Bioactivity-guided fractionation led to the isolation of afzelin and quercetin 3-O-D-arabinopyranoside, as inhibitors of acyclovir-resistant HSV-1 [11].

The safety of avocado has been demonstrated in a study in which 2.5 g/kg (p.o) per day of the aqueous seed extract of P. americana, in a sub-acute experiment, were administered to the rats for 4 weeks [12]. Although, avocado like other herbal products is safe and generally better tolerated than synthetic medications, there is limited scientific evidence to evaluate different side effects because of contaminants, or interactions with drugs. Besides, further studies need to be accomplished on the metabolic effects of different parts of avocado for other possible mechanisms which is assayed and proved in the several studies [13].

2. Materials and Methods

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

2.1 Preparation of PAME and PAWE

Persea Americana were purchased from local market and it was extracted by using phosphate buffer using Soxhlet extraction method. The finally obtained extracts were termed as PAME and PAWE respectively and it utilized for further assays.

2.2 Preliminary phytochemical screening of PAME and PAWE

PAME and PAWE were screened for terpenoids, phytosterol, tannin, phenolic, glycoside, Saponin, flavonoid, carbohydrates, proteins, steroids and lipids [14].

2.3 Reverse Phase High Performance Liquid Chromatography analysis of PAME and PAWE

PAME and PAWE 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 [15].

2.4 Antimicrobial assay of PAME and PAWE

The bacterial cultures (E. coli, Salmonella, Pseudomonas, Shigella 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 37mg and 44mg of PAME and PAWE respectively in 1.0mL of methanol to get 37mg/mL and 44mg/mL concentration respectively followed by sonication for 2min. The 100µL of this test solution containing 3700µg and 4400µg of PAME and PAWE respectively were added into the respective wells (3-10mg). The standard antibiotic drug Amoxycillin was kept as positive control and tested against all the pathogens. These plates were incubated at 37 °C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [16]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported

2.5 ICP-OES analysis of PAME and PAWE

PAME and PAWE 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 [17].

2.6 GC-MS analysis of PAME and PAWE

PAME and PAWE were analysed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Impact Ionisation Total Ion Chromatography (EITIC) mode with a 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 a 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 [18].

2.7 Direct hemolytic activity of PAME and PAWE

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 PAME and PAWE (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 [19]. 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

3. Results and Discussion

3.1 Chemical Characterization of PAME and PAWE

PAME and PAWE were found to be present of terpenoids, Glycosides, flavonoids, carbohydrates and steroids. But only PAME withholds polyphenol compounds, and PAWE contains only saponins, respectively, in the extracts when it is subjected to preliminary phytoconstituent characterization (Table 01). PAME and PAWE show the presence of several minerals like Aluminium, cadmium, zinc, barium, boron, etc., (Table 02).

Table 1: PAME and PAWE preliminary phytoconstituent characterization

Phytochemical	PAME	PAWE
Terpenoid	+ ve	- ve
Phytosterol	-ve	-ve
Tannin	-ve	-ve
Phenolic	-ve	-ve
Glycosides	-ve	+ve
Saponin	-ve	-ve
Flavonoids	-ve	-ve
Carbohydrate	+ve	+ve
Protein	-ve	+ve
Steroids	+ve	-ve
Lipids	+ve	-ve

Table 2: PAME and PAWE Aluminium, cadmium, zinc, barium, boron, etc.,

Metals	PAME (ppm)	PAWE (ppm)
Aluminum	0.09	0.04
Boron	0.17	0.11
Barium	0.02	0.08
Cadmium	0.001	0.007
Chromium	0.004	0.001
Copper	0.045	0.051
Iron	0.08	0.98
Manganese	0.03	0.06
Molybdenum	0.00	0.01
Nickel	0.011	0.019
Lead	0.00	0.08
Zinc	0.12	0.18

3.2 RP-HPLC analysis of PAME and PAWE

PAME and PAWE HPLC chromatograms confirm the presence of 06 and 11 different types of compounds by eluting 06 and 11 peaks at different retention times, respectively, in reverse-phase HPLC attached to a Variable Wavelength Detector. The sample was eluted at 216nm at room temperature (Fig. 01 and Fig. 02).

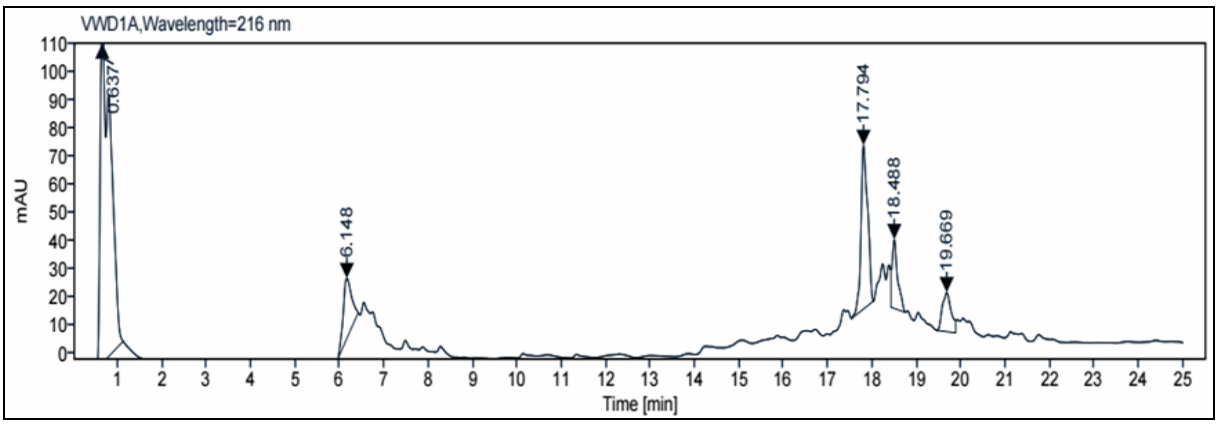

Fig 1: HPLC Chromatogram of PAME

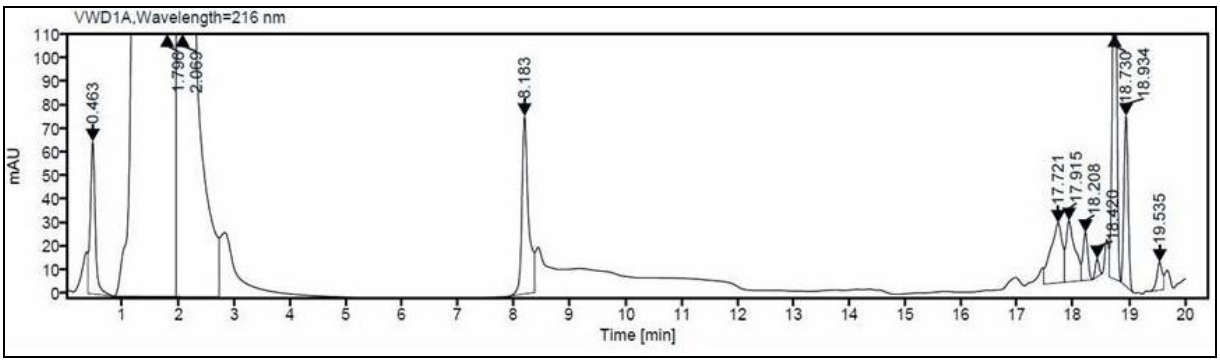

Fig 2: HPLC Chromatogram of PAWE

3.3 GC-MS analysis of PAME and PAWE

PAME and PAWE were found to exhibit 08 and 16 different sets of compounds, respectively as per GC-MS analysis respectively (Fig.03 and Fig.04).

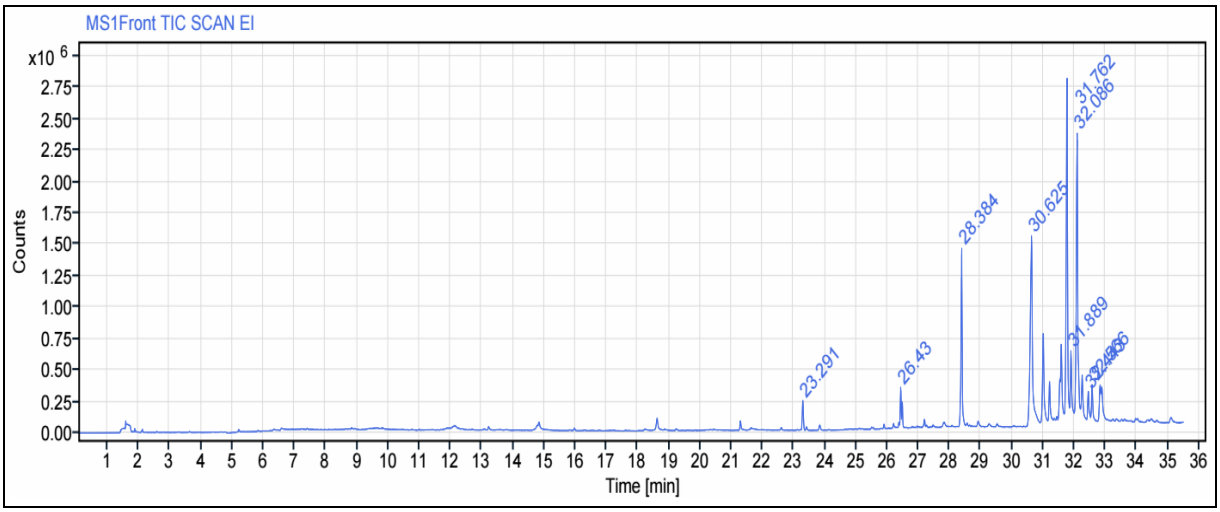

Fig 3: GC-MS Chromatogram of PAME

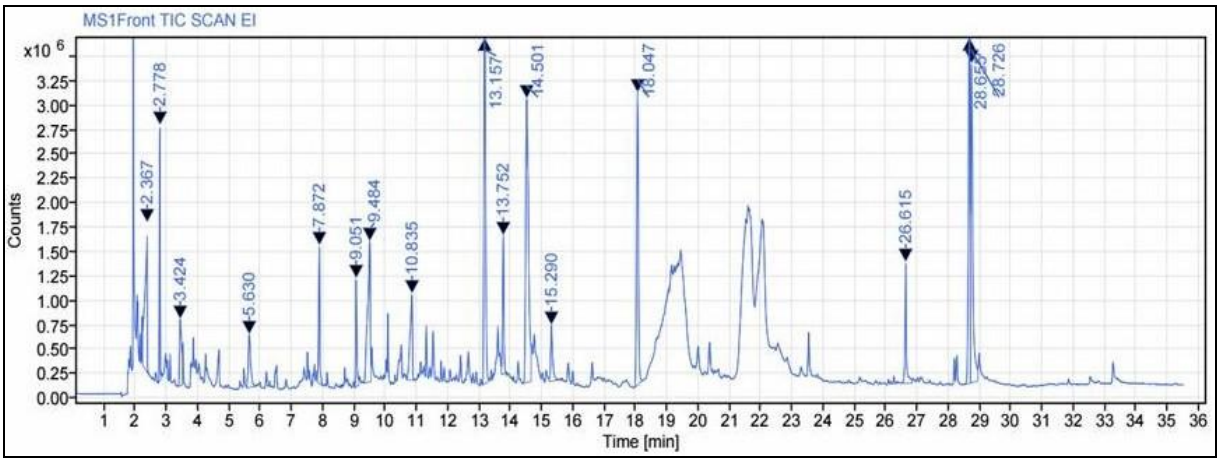

Fig 4: GC-MS Chromatogram of PAWE

3.4 Non-toxic property of PAME and PAWE

Moreover, PAME and PAWE did not hydrolyze RBC suggested its nontoxic property (Fig.05 and Fig.06).

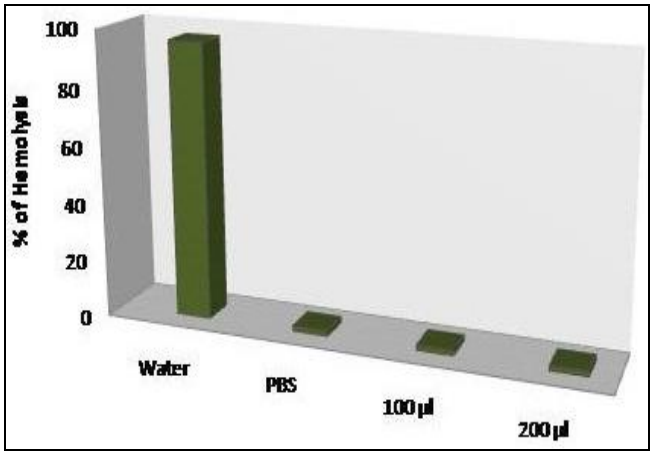

Fig 5: Hemolytic activity of PAME

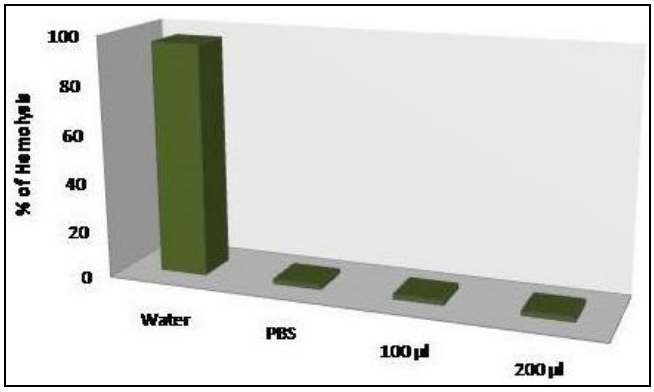

Fig 6: Hemolytic activity of PAWE

3.5 Antimicrobial activity of PAME and PAWE

PAME and PAWE antimicrobial property were performed with E. coli, Salmonella, Pseudomonas, Shigella and S. aureus. PAME and PAWE found to show zone of inhibition against all five microbial cultures (Fig.07 and Fig.08).

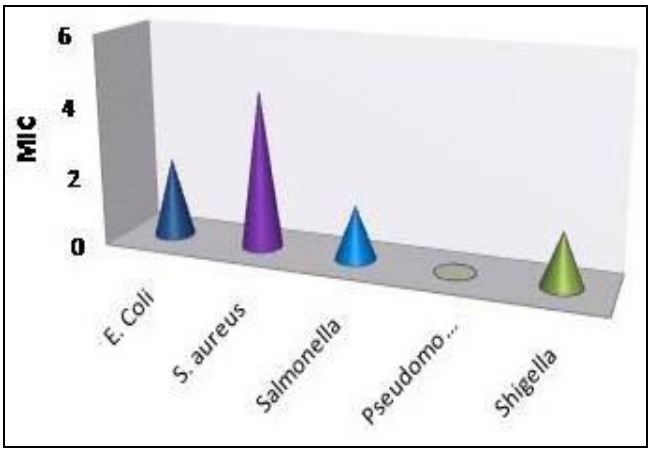

Fig 7: Anti-microbial property of PAME

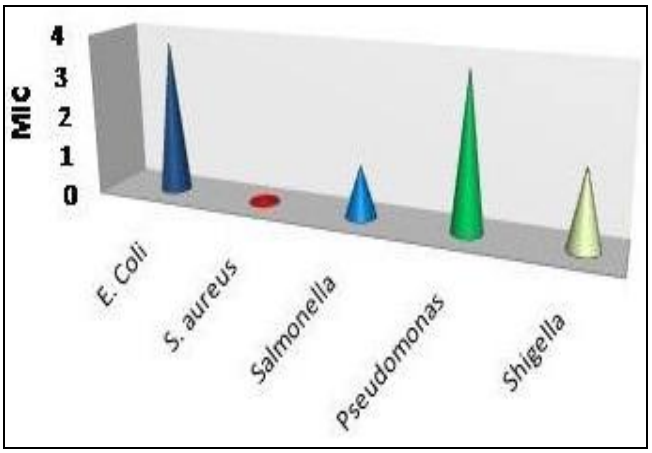

Fig 8: Anti-microbial property of PAWE

4. Conclusion

In conclusion, this study demonstrates the preliminary characterization of PAME and PAWE and its found that it is exhibited antimicrobial property and weak anticoagulant property with its non-toxic nature.

5. Acknowledgments

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6. Declaration of Conflict of Interest

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

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