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Article

Microencapsulation and Characterization of Natural Polyphenols from PHF Extract

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Abstract: Microencapsulation of polyherbal formulation (PHF) extract was carried out by freeze drying method, by employing gum arabic (GA), gelatin (GE), and maltodextrin (MD) with their designated different combinations as encapsulating wall materials. Antioxidant components (*i.e* total phenolic contents (TPC),Total flavonoids contents (TFC), and total condensed contents (TCT)), antioxidant activity (*i.e.* DPPH, β-carotene & $ABTS^+$ assays), moisture contents, water activity (aw), solubility, hygroscopicity, glass transition temperature (T_g), particle size, morphology, *in vitro* alpha-amylase and alpha-glucosidase inhibition and bioavailability ratios of the powders were investigated. Amongst all encapsulated products, T_B (5% GA and 5% MD) and T_C (10% GA) has proven to be the best treatments with respect to the highest preservation of antioxidant components and their antioxidant potential by DPPH and β-carotene assays and noteworthy for an $ABTS^+$ assays, in addition, the aforesaid treatments also demonstrated lower moisture content, aw, particle size and higher solubility, hygroscopicity and glass transition temperature (T_g). All freeze dried samples showed irregular (asymmetrical) microcrystalline structures. Furthermore, T_B and T_C also illustrated the highest *in vitro* anti-diabetic potential due to great potency for inhibiting alpha-amylase and alpha-glucosidase activities. In the perspective of bioavailability, T_A, T_B and T_C demonstrated the excellent bioavailability ratios (%).Futhermore, the phytochemo-profiling of ethanolic extract of PHF was also revealed to find out the bioactive compounds.

KeyWords: Microencapsulation; polyphenols; freeze-drying; antioxidant activity; *in vitro* dialyzability; *in vitro* anti-diabetic potential.

1. Introduction

Diabetes mellitus (DM) is the endocrine metabolic disorder characterized by increased blood glucose level coupled with abnormality in protein, carbohydrate and fat metabolism. It has become major disorder which affected 346 million people worldwide and this number is expected to be double by the year of 2030 [1, 2]. It is such a progressive endocrine disorder of glucose metabolism that eventually leads to micro- and macro-vascular changes causing secondary complications that are incredibly challenging to manage [3]. Clinically, DM can be categorized into two types; Type-I DM,

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arisen due to the inadequate synthesis of insulin by β-cells of the pancreas and featured with complications *i.e.* **diabetic retinopathy,** neurodegenerative, kidney-related issues **etc. it is an** insulin-dependent DM, whereas, type-II DM; (*i.e.* non-insulin-dependent) is primarily characterized by insulin resistance (*i.e.* a condition in which peripheral cells do not respond normally to insulin) or β-cell dysfunction which eventually leads to diabetes-associated cardiovascular (CVD) and fatty liver disease [4]. Diverse and multifaceted factors have played pivotal role for onset and progression of DM, including unhealthy foods, auto-immune disorders, ecological factors and miscellaneous variables [5-8]. However, "oxidative stress", the ultimate outcome of reactive oxygen species (ROS) and nitrogen oxide (NO), has been considered as one of the predominant factors as reported by many studies [9-11].

To manage these ROSs, all living cells have intrinsic antioxidant manufacturing system, which might helpful to retain the redox condition of the body at cellular and sub-cellular level by neutralizing the reactive oxygen species (ROSs). The loss in balance between the free radical production *i.e.* high ROSs production and biological system's ability to produce endogenous antioxidants can lead to a aforementioned health-related disease called "oxidative stress" [12]. The excess production of these ROSs are considered lethal for human health as their surplus generation can leads to different patho-physiological conditions like fast aging process via damaging the nucleic acids and changing in the conformation of proteins, heart-related disorders (*i.e.* CVDs), diverse types of cancers, immunity related dysfunctions, inflammation, membranous lipid oxidation, decline of hydroperoxide synthesis, neurodegenerative disorders, lungs and kidney illness, UV- irradiation, and osteoporosis/ bone- related diseases. Direct correlation between oxidative stress and insulin resistance (key factor for type-II DM) has been elaborated in mini review by Hurrle et al. [13].

Nowadays, research has been focused in exploring naturally occurring antioxidants to circumvent the complexities due to oxidative stress. Diverse types of herbs, spices, teas, flowers, seeds, fruits, vegetables and marine foods are now considered for the expected antioxidant excellent sources to replace synthetic antioxidants [14-16]. Amongst naturally-occurring antioxidants, polyphenols and their derivative compounds represented a diverse class of ubiquitous material *i.e.* from simple molecules to complex configuration such as phenolic acids; hydroxybenzoic and hydroxycinnamic acids, hydrolyzable and condensed tannins, and flavonoids, these are most important compounds for nutraceutical, therapeutics and pharmacological point of view [14, 17] and revealed various health endorsing activities: antioxidant activity via free radicals scavenging, declining of hydroperoxide development, hampering the lipid oxidation, anti-diabetic, anti-malarial, anticancer activity etc [14, 15].

Recently, polyherbal formulations (PHF)/ herbal nutraceuticals are considered as a great source of natural polyphenols all over the globe due to their dynamic medicinal and therapeutic claims.

Previous investigations illustrated that selected individual plants contained abundant quantity of polyphenols and their herbal combinations were found to produce best antioxidant activity among all individual extracts due to synergistic effect. Synergism played a vital role via two different kind of mechanism in context of interaction *i.e.*, pharmacokinetic (PK) and pharmacodynamics (PD) [18]. In the provisions of pharmacokinetic synergism, capacity of herbs/plant to aid in the absorption, delivery, metabolism and elimination of the other herbs from the body was overlooked. Whilst in pharmacodynamic synergism mechanism, assessment the synergistic effect when active components with analogous therapeutic prospective are targeted to an interrelated physiological structure/receptor. Here, combination of herbs may work on multiple targets at the same time to offer a comprehensive relief [19]. Owing to synergism, polyherbal formulation (PHF) demonstrated vast advantages over single herbal formulation (SHF) likewise: superior restorative effect can be attained with a polyherbal formulation (PHF); to acquire enviable pharmacological accomplishment low dosage would be required, consequently lessening the risk of harmful side effects. Additionally, PHF facilitate the patient's convenience by eradicating the need of taking more than one formulation at a time, which ultimately leads to better compliance and therapeutic effect. All the aforesaid advantages have outcome in the attractiveness of PHF in the marketplace when compare to SHF [20].

Polyphenols are incredibly sensitive in diverse range of circumstances, during food processing and storage practice likewise; high temperature of surrounding, incidence of oxygen and light, pH, existence of oxidative enzymes, moisture contents [21]. The degradation of natural antioxidants may hamper the possible effectiveness of application of these antioxidants in food /nutraceutical and pharmaceutical applications and commercially available anti-diabetic drugs also produce unconstructive effects on other metabolisms [22], so supplementation of anti-hyperglycemic substances, which also possess antioxidant properties, might be an alternative therapy to overcome this critical condition [23, 24]. To address these shortcomings and to augment the antioxidant stability and preserve their diverse bioactivities including anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic capabilities, the microencapsulation has been employed successfully as a reliable technique to circumvent the unwanted degradation of bioactive compounds, shielding them from adverse environmental circumstances. Furthermore, various type of wall material has been used for microencapsulation procedure, but cost effectiveness and physico-chemical distinctiveness must be considered, including: hygroscopicity, biodegradability, emulsifying feature, adaptability to gastrointestinal tract (GT), viscosity, solids content [25].

At present, the preferred wall materials for microencapsulation for various fruit juices and plant/herbs extracts are maltodextrins (MD), gum arabic (GA) and gelatin (GE) [26]. Maltodextrin of various dextrose equivalents (DE) are generally used as wall material owing to their distinct characteristics likewise; low viscosity, high solubility in water and their solutions are monochromic in appearances. These features made them frequently used carrier/wall materials in the micro-encapsulation procedure. Gum Arabic (GA) (exudates of acacia), owing to its unique features *i.e.* naturally colorless, low viscosity, high retention of volatiles and ability to make stable emulsion is ultimately considered as excellent encapsulating agent whereas its high economic cost provoked researcher for full or partial replacement of the encapsulation agent [26-28]. In addition, gelatin (GE), is also a better option for microencapsulation because of its superior characteristics for emulsification, film-formation, water solubility, last but not least ability to form finer dense complex**.** According to Fang and Bhandari [29], a sole microencapsulating agent has limitation over all required attributes to improve microencapsulation effectiveness, eventually has been resolved by using different combination of polymers due to their diverse features. The selection for polymer's combinations which possibly consequence in superior microencapsulating efficiency and regarded economically suitable than the single biopolymers has been becoming the point of emerging interest [29, 30].

In the current study, polyherbal formulation (PHF) was firstly made with equal ratio of roots of *Chlorophytum borivilianum,* roots of *Astragalus membranaceus,* roots of *Eurycoma longifolia,* and seeds of *Hygrophila spinosa* T. Anders having previously proven diverse ethno-pharmacological applications [31-34] as polyphenols enriched nutrient supplement, then PHF extract was further microencapsulated by freeze drying method using different wall materials, subsequently antioxidant components (*i.e.* TPC, TFC, and TCT), antioxidant activity (*i.e.* DPPH, β-carotene &ABTS^{+} assays), anti-diabetic potential (*i.e.in vitro* alpha-amylase and alpha-glucosidase inhibition) physical property like; moisture contents, water activity (aw), solubility, hygroscopicity, glass transition temperature (T_g), morphological characteristics (*i.e.* particle size, morphology), and bioavailability ratios of the microencapsulated powders were investigated. In last, the chemo-profiling for ethanolic extract of PHF was also studied.

2. Materials and Methods

2.1. Materials, chemicals, reagents and encapsulating agents

All different parts of herbs (detail in Section 3.2) were purchased from Faisalabad-Pakistan and their identification and respective characteristics were authenticated by Prof. M. Jafar Jaskani from Institute of Horticulture, University of Agriculture Faisalabad (UAF) Pakistan. All chemicals used were of analytical grade or higher where suitable. DPPH (2, 2-diphenyl-1-picryl-hydrazyl), Foline-Ciocalteu (FC), β-carotene, Butylated hydroxyltoluene (BHT), TWEEN 20, quercetin, Sodium

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carbonate, ABTS (2, 20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid), α-tocopherol, Linoleic acid, (+)-catachin, quercetin, $AlCl_3.6H_2O$, HCl, Vanilline, NaOH, Potassium persulfate, Trolox, gallic acid were purchased from Sigma-Aldrich GmbH (Sternheim, Germany). Alpha-amylase from porcine pancreas, alpha-glucosidase from *Saccharomyces cerevisiae*, paranitrophenyl-glucopyranoside, pepsin (porcine-7000), bile salts pancreatin (p-1750), piperazine-NN-bis (2-ethane-sulfonic acid) di-sodium salt (PIPES), gelatin (GE), HPLC-grade methanol, acetonitrile ethanol, acetone were supplied by Sigma-Aldrich (USA), soluble starch (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Ultra-pure water (18 MΩ cm−1) was acquired from Milli–Q purification device (Millipore Co. USA). Sodium hydrogen carbonate was purchased from Merck (Germany). Sea sand was of 200-300 grain size from Scharlau (Barcelona, Spain). The encapsulating agents were: gum arabic (GA) (Sangon Biotech, Co. China), maltodextrin (MD) (Dextrose equivalent of 12) was purchased from Corn Products (Cabo de Santo Agostinho, Pernambuco, Brazil)

2.2. Polyherbal formulation (PHF)

Polyherbal formulation (PHF) was made by combining the root of *Chlorophytum borivilianum* roots of *Astragalus membranaceus,* roots of *Eurycoma longifolia*, and seeds of *Hygrophila spinosa* T. Anders, in a ratio of 1:1:1:1 respectively.

2.3. Preparation of sample

Firstly, the roots and seeds of aforesaid herbs were cut into small pieces, followed by thorough washing with deionized water in order to avoid any contamination. The PHF material was then dried for 12 days in dark in well ventilated room at room temperature (23±8), and subsequently grounded with mortar and pestle to make crude powder with the help of liquid nitrogen, until a uniform sieve size equivalent to (1.0 mm) was achieved. The resulting powder was stored at -80°C in inert vacuum bags until used for extraction as followed.

2.4. Pressurized liquid extraction (PLEx)

PLEx was executed in a Dionex ASE 350 system (Dionex, Sunnyvale, CA) with the powder of PHF obtainedas mentioned above. Aliquot of 5.0g of powder of PHF was mixed with diatomaceous earth (1/1) and placed in a 34mL stainless–steel cells. The extraction was performed *via* 3 consecutively applied steps with absolute solvents of increasing polarity, in order to get the maximum possible number and amount of secondary metabolites of various polarities and miscibilities, namely, acetone, ethanol, methanol and their aqueous mixtures with water (1:10, 3:10), and pure water. Extraction time was of 22 minutes; pressure 10.6 MPa; temperature 75 °C (for acetone, ethanol and methanol) and 135 °C (for water). Organic solvents were removed in a rotary vacuum evaporator at 38 °C, while the residual water was removed in a freeze drying unit. The extracts after solvent evaporation were placed under nitrogen flow for 20 min and stored in dark glass bottles at −80 °C until analyzed.

2.5. Development of microencapsulated powder products

In order to prepare the particular dispersions, 100mL of PHF aqueous extract was mixed individually with different preselected combination of microencapsulating wall materials as follow: A (5% GA and 5% GE) (hereafter referred and discussed as T_A); B (5% GA and 5% GE) (hereafter referred and discussed as T_B), C (10% of GA)(hereafter referred and discussed as T_C), and D (10% of MD) (hereafter referred and discussed as T_D),under constant shaking with 220 rpm, at 35°C for 30 min by a shaking unit (Shanghai, CIMO). Afterward, these dispersions/emulsions were microencapsulated through lyophilization process for formulating four distinctive treatments. *i.e.* T_A, T_B, T_C, T_D.

For microencapsulation by means of freeze-drying process, the above-mentioned dispersions/emulsions were kept at −20°C (freezer) for 48 h. Subsequently, the samples were placed in lyophilization unit (Labconco, Germany) for freeze drying at −56.5°C, with vacuum pressure of

20μmHg for 60 h. After the completion of freeze drying process, the samples were crushed utilizing a mortar and pestle assembly. Finally, the desirable final microencapsulated products were sealed in polyethylene bags and aluminum pouches as well and stored in desiccator encompassing silica until further analysis.

2.6. Determination of bioactive compounds and their bioactivities after microencapsulation

Bioactive components which were determined after the microencapsulation were total phenolic compounds (TPC), total flavonoids compounds (TFC), total condensed tannins (TCT). While the bioactivities of the microencapsulated powders were measured in terms of total antioxidant activity determined by β-carotene bleaching assay (TOAA), $ABTS^{+}$ radical scavenging activity, and DPPH scavenging capacity. All these spectrophotometric analysis were performed according to previously developed methods with minor alteration [16, 35, 36]. The results for $ABTS^{+}$ radical scavenging activity are deliberated as EC_{50} values (mg of extract/mL) for comparison. Effectiveness of antioxidant properties is inversely correlated with EC_{50} value.

2.7. Determination of the physical properties of the microencapsulated powders

2.7.1 Moisture content

The moisture contents of the microencapsulated products were estimated by using the method describes in manual AOAC [37], *i.e.* by calculating the loss of sample after weight after heat up at 105^{0}C.

2.7.2 Water activity (Aw)

The water activity (A*w*) of all lyophilized samples was calculated through the direct analysis in electronic meter (Aqualab Dew Point 4TEV, USA), to gain the constant state the samples were firstly placed at 25°C for at least 15 mints.

2.7.3 Solubility

The solubility of microencapsulated products was measured by the method described by Cano-Chauca et al. [38], with minute alterations. The sample's quantity of 1.0g was mixed up with 100mL distilledwater in beaker and stirred with magnetic stirrer(MS-H-S10) for 20mints. After that the centrifugation of solution carried out at 3000×g (Thermo Scientific) for10mints. The quantity of 25mL of the supernatant was transferredto a petriplates (pre-weighted) and dried in oven at 105^{0}C for 4.0 h.The solubility was measured as a result of weight difference and demonstrated in the term of percentage (%).

2.7.4 Hygroscopicity

For the estimation of the hygroscopicity, the encapsulated powder of 1.0 g was placed in dessicator with saturated NaCl solution (74.6%) at temperature of 25^{0}C. After 1 week, samples were weighed and hygroscopicity was represented in the term of percentage (%) [39].

2.7.5 Glass transition temperature (Tg)

The glass transition temperature (Tg) of the microencapsulated products was calculated by means of differential scanning calorimetry (DSC) (DSC-2000-New Castle, DE). The weight of 7-8mg of sample was placed in aluminum hermetic pots. For the reference purpose, a aluminum pan without sample was used. Ultra-pure nitrogen N_2 was used as purge gas (flow rate 50 mL/min). The temperature ranged from -80 ^{0}C to 120 ^{0}C at a heating rate of 40 ^{0}C/ min. The glass transition temperature was determined by utilizing software of TA Universal Analysis 2000.

2.8. Morphology and size distribution

The configuration of micro-particles obtained from diverse encapsulating wall material and their combinations were examined by scanning electron microscope (Quanta 250 EFI). At first, very minute was fixed on surface of double sided tape of carbon then finally evaluated the samples under microscope with 400 X magnification. The analysis for particle size distribution average and particle size was conducted by the means of ImageJ (NIH, Bethesda, MD).

2.9. In vitro Assays

2.9.1. Alpha-Amylase Inhibition Assay

The inhibition of alpha-amylase was determined using an assay modified from the Worthington Enzyme Manual [40]. Aliquot 0 - 4 mg/ml in DMSO (v/v 1:1) of each encapsulated PHF samples was prepared and 500 µl of each sample were mixed with 500 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/ml) and incubated at 25°C for 10min. After pre-incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 15 ml of distilled water, and the absorbance was measured at 540 nm using a micro-plate reader (Thermomax, Molecular device Co., Virginia, USA). The experiments were performed in duplicate and the absorbance of sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extract) were also recorded. The absorbance of the final each encapsulated PHF sample was obtained by subtracting its corresponding sample blank reading. Acarbose was prepared in distilled water and used as positive controls

The percentage inhibition was calculated using the formula;

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}\ 100$$

Where Ac and Ae are the absorbance of the control and extract, respectively

IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of each encapsulated PHF samples was prepared were determined by plotting graph with varying concentrations of the plant extracts against the percent inhibition.

2.9.2. Alpha-Glucosidase Inhibition Assay

The alpha-glucosidase was assayed using a method modified by Apostolidis et al. [41]. Aliquot of 0 - 4 mg/ml in DMSO (v/v 1:1) of each encapsulated PHF samples were prepared. 50 µl of each concentration sample was mixed well with 100 µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/ml) and the mixtures were then incubated in 96-well plates at 25°C for 10 min. After pre-incubation, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Before and after incubation absorbance readings were recorded at 405 nm using a micro-plate reader (Thermomax, Molecular device Co., Virginia, USA) and compared to a control which contained 50 µl of the buffer solution instead of the extracts. The experiments were performed in triplicate and the α-glucosidase inhibitory activity was expressed as percentage inhibition. Acarbose was prepared in distilled water and used as positive controls. The percentage inhibition was calculated using the formula;

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}\ 100$$

Where Ac and Ae are the absorbance of the control and extract respectively.

IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of each encapsulated PHF samples was determined by plotting graph with varying concentrations of the plant extracts against the percent inhibition

2.9.3. Determination of Bioavailability of microencapsulated products by in vitro dialyzability assay

The estimation for bioavailability of all microencapsulated products was determined by the method developed by Pineiro et al. [42].

2.10. Acute toxicity

The acute oral toxicity study was carried out in compliance with Organization for Economic Cooperation and Development (OECD) guideline 425 [43]. All mice (n=5) for testing were fasted for 12 h and weigh have been recorded and subsequently received the solution of microencapsulated products of PHF at the final concentration of 2000 mg/kg by gavage. The animals were observed individually at least once during the first 30 min after dosing, periodically for first 24 h and regularly thereafter for 14-day of feeding period for gross behavioral changes, toxicity symptoms or mortality.

2.11. LC-ESI-QTOF-MS analyses

For LC-ESI-QTOF-MS analysis, firstly ethanolic extract was prepared using PLEx as described in Section 3.4. Afterwards obtained ethanolic extract was used to for the metabolite profiling of PHF using an Agilent 1100 Liquid Chromatography system (Agilent Technologies, Palo Alto, CA, USA) furnished with a standard auto-sampler. The analytical column used was characterized as Phenomenex Gemini C18 (3µm, 2 x 150 mm) operated at 25ºC with a gradient elution portfolio at a flow rate of 0.2 ml/min. The mobile phases used were of acidified water (0.5% acetic acid) (A) and acetonitrile (B). The following multi-step linear gradient applied in following fashion: 0 min, 5% B; 5 min, 15% B; 25 min, 30% B; 35 min, 95% B; 40 min, 5% B. The initial conditions were maintained for 5 min. The injection volume of sample in system was 1µl. The LC-MS system was further composed of a Dionex Ultimate 3000 Rapid Separation LC system coupled to a micrOTOF QII mass spectrometer (Bruker Daltonics, Bremen, Germany) fitted with an electro-spray source operating in positive mode. The LC system contained an SRD-3400 solvent rack/degasser, an HPR-3400RS binary pump, a WPS-3000RS thermostated auto-sampler, and a TCC-3000RS thermostated column compartment. The micrOTOF QII source parameters were as follows: temperature, 200°C; drying N_2 flow, 8 L/min; nebulizer N_2, 4.0 bar; end plate offset, −500 V; capillary voltage, −4000 V; mass range, 50−1500 Da, acquired at 2 scans/s. Post acquisition internal mass calibration used sodium formate clusters with the sodium formate delivered by a syringe pump at the start of each chromatographic analysis. Nitrogen was used as drying, nebulizing and collision gas. The precise mass data of the molecular ions were processed using Data Analysis 4.0software (Bruker Daltoniks), which delivered a list of potential elemental formulas via the Generate Molecular Formula Editor. The generate molecular formula Editor uses a CHNO algorithm, which deals with standard practicalities such as electron configuration, minimum/maximum elemental range and ring-plus double-bond equivalents, as well as a sophisticated comparison of the theoretical with the measured isotope pattern(Sigma Value) for increased confidence in the recommended molecular formula. The commonly acknowledged accuracy threshold for validation of elemental compositions was established at 5 ppm (Bringmann et al., 2005). It is significant to point out that even with very high mass precision (<1ppm) many chemically likely formulas may be found, subjected to the mass regions considered and so high mass accuracy alone is not enough to discount enough candidates with complex elemental compositions. The use of isotopic abundance patterns as a single further constraint, however, eliminates>95% of the false candidates. This orthogonal filter can diminish numerous thousand nominees down to only a small number of molecular formulas. During the development of the HPLC method, the instrument was calibrated externally with a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, Illinois, USA) directly linked to the interface and injected with a sodium acetate cluster solution containing 5mM sodium hydroxide and 0.2% acetic acid in water: isopropanol (1:1, v/v). The calibration solution was injected at the beginning of each run and all the spectra were calibrated prior to compound identification. By using this method, an exact calibration curve based on several cluster masses, each differing by 82 Da (NaC2H3O2) was obtained. Due to the compensation of temperature drift in the

micrOTOF-Q II, this external calibration provided accurate mass values of better than 5 ppm for a complete run without the need for a dual sprayer setup for internal mass calibration.

3. Statistical Analysis

All statistical analyses were conducted using a one-way analysis of variance using Dunnett's comparison tests or unpaired t-tests. All calculations were carried out using GraphPad Prism 5 (GraphPad Software, San Diego CA, www.graphpad.com). Significance was observed at $p < .05$.

4. Results and discussion

4.1. The effect of microencapsulation on the contents of antioxidant components and antioxidant activity of PHF

The contents of antioxidant components of PHF extract treated with different encapsulating wall materials were shown in Table 1. In comparison to untreated extract, all microencapsulated treatments have less antioxidant components (*i.e.* total phenolic contents (TPC), total flavonoids contents (TFC), and total condensed tannins (TCT). The retention for all freeze-dried treatments demonstrated in the term of percentages, ranged from 94.28% to 68.22% for TPC, 76.46% to 40.35% for TFC and 79.24% to 59.70% for TCT representing the effectiveness of microencapsulation procedure. The wide-ranging powders produced from the microencapsulation process, especially those obtained from the treatment C (T_C), retained higher contents of antioxidant components. In general, these results may be associated with the type and concentrations of different wall materials. There were many multifaceted factors which were responsible for hammering of polyphenol compounds during freeze drying method, the crushing of lyophilized microencapsulated products after freeze-drying, was considered one of the key factor which may cause the degradation of bioactive components in the final products by boosting the product's contact with environment. Our finding was in agreement with previous work in which authors explored that lyophilized wine product contained almost 70% of the original phenolics components [44-45]. Other factors which may responsible for declining the concentration of active components include: formation of microspheres during the lyophilization due to a scattering of the bioactive components inside the configuration of encapsulating wall materials *i.e.* consisting of one or more constant phase of encapsulating agents [46], development of micro-pores in the aforesaid microspheres, mainly associated to sublimation process during lyophilization [47]. In the current study, lyophilized product encompassed a reduction of 5.72–31.78% for total phenolics contents (TPC), declined trend of 23.54–59.65% and 20.76-40.30% was also observed for TFC and TCT respectively. Despite the reduction of antioxidant components of microencapsulated products, a significant retentions were also observed (described above in detail with percentages) comparable/higher to prior studies *i.e.* authors found, that acai pulp microencapsulated with GA have phenolic retention of 94.1% [26].

The freeze dried product microencapsulated with 10% GA demonstrated (T_c) the exceptional conservation for antioxidant components (*i.e* TPC, TFC, TCT). The order of effectiveness of microencapsulation for other remaining treatments was as followed: $T_B > T_A > T_D$. The higher competence of T_C treatment was mainly attributed to the structure of gum arabic (GA), because it is a hetero-polymer made up of dense branches of sugar, containing a minute quantity of protein which connected to the carbohydrate skelton via covalent bonds, proceeding as a tremendous microencapsulating material [48]. Noteworthy results were also found for T_B and T_A, which might be credited to presence of 5% GA. In contrary, no significant difference was noticed for the lyophilized product having10% MD as wall material (T_D).

The antioxidant activity for microencapsulated powders determined by DPPH, β-carotene and $ABTS^+$ assay were illustrated in Table 1. All microencapsulated products had showed decrease antioxidant by DPPH assay in relation to original extract (control) and their retention ranged from 38.84% - 64.50%. Treatment B (5% GA and 5% MD) and treatment C (10% GA) illustrated the highest antioxidant activity; these results were agreement with previously found values by Souza et al. [49]. The order for effectiveness was noticed as: $T_B > T_C > T_A > T_D$. In the case of β-carotene bleaching assay,

the antioxidant retention for all microencapsulated products were explored from 77.59 to 93.93% in comparison to original extract, T_B (5% GA &5% MD) showed maximum value for antioxidant activity in a similar way as in DPPH assay. Remaining treatments has been categorized in context of efficacy as followed: $T_C > T_D > T_A$. Referring to antioxidant assay by $ABTS^+$ radical scavenging activity, the range of retention was from 62.2% to 86.68%. The noteworthy consequence was revealed for T_A (5% GA & 5% GE), while T_B (5% GA and 5% MD) and T_C (10% GA) also illustrated the significant results with retention of 75.27% and 74.18% respectively. The above discussion suggested the worthiness of diverse antioxidant assay for secure and overwhelming conclusion, because each assay comprised its own preciseness and proceeds at a challenging site of action. Amongst the all lyophilized encapsulated products, the antioxidant activity was higher in T_B and T_C, being related to the presence of high antioxidant components (*i.e.* TPC, TFC &TCT) (Table 1), which provided an excellent defense system against unrestrained oxidation, owing to its high reducing power. Furthermore, there is no report yet on microencapsulation of aforesaid polyphenol enriched extract from PHF and their characterization related to analysis for antioxidant.

4.2. Physical characteristics of microencapsulated powder products

Physical factors *i.e.* water activity; moisture contents and hygroscopicity are indispensable for encapsulating products steadiness and storage, whilst aqueous solubility is correlated with ability of powder products for reconstitution [28].

The moisture contents for four different lyophilized encapsulated products were demonstrated in Figure1A. The moisture content of said powders was ranged from 7.07% to 9.04%; on the contrary, no significant difference was found between T_B and T_D (7.41% and 7.21%, respectively). Our findings was validated by earlier investigation which elaborated the moisture contents for blackberry fruit drink encapsulated by means of MD and trehalose dehydrate were of 2.44–6.11% [50]. Lower freezing temperature *i.e.* less than -40°C consequence in quick freezing, eventually caused tiny pores in the superficial coatings, which might encumber the mass transfer and regarded as an obstacle for sublimation process, causing the higher retention of moisture contents in microencapsulated products [51].

The water activity (aw) of all microencapsulated products (Figure 1B) was ranged from 0.310 to 0.450, and all final encapsulated products were noticeably dissimilar from one another, apart from T_B (5% GA and 5%MD). T_D (10% MD) demonstrated the maximum a_w value of 0.450 which was corroborating with previous study carried out by Gurak et al. [52] who found that a_w of grape fruit drink microencapsulated by the means of maltodextrin (MD) utilizing lyophilization technique was 0.430.

Various factors that determine the solubility of the microencapsulated powdered products includes: the feed composition and particle size. The selection of the wall material is very important, not only for the solubility itself but also to the crystalline state that ultimately bestowed to the dried powders [53]. The aqueous solubility for all lyophilized treatments was ranged from 84.06 to 92.31% as illustrated in Figure1C. The solubility of the final product possibly not only associated with solubility prospective of microencapsulating wall material but also on attainted particle size in final desirable product; if particle size would be minute, it would ultimately provide the better surface area's availability for the hydration process [54, 55]. The highest solubility value was obtained for treatment T_D (10% MD) that was consistent with previous work. Moreira et al. [30] elaborated the solubility percentage for acerola pomace extract ranged from 90.97 to 96.92%, using MD and tree's gum of cashew apple as microencapsulating wall materials.

The hygroscopicity values for all microencapsulated powder products by the means of freeze-dying method were depicted in Figure 1D. These were ranging from 11.92% to 14.35%, representing a lesser amount of hygroscopicity values for powder products; hence assisted the protection of antioxidant components. The findings of current work have much resemblance with preceding work, utilizing related sort of microencapsulating wall materials. Some renowned investigators reported the hygroscopicity of microencapsulated products made up from bark extract of jaboticaba tree using MD and GA as wall material of 17.75%. The lyophilized powdered products demonstrated the lesser

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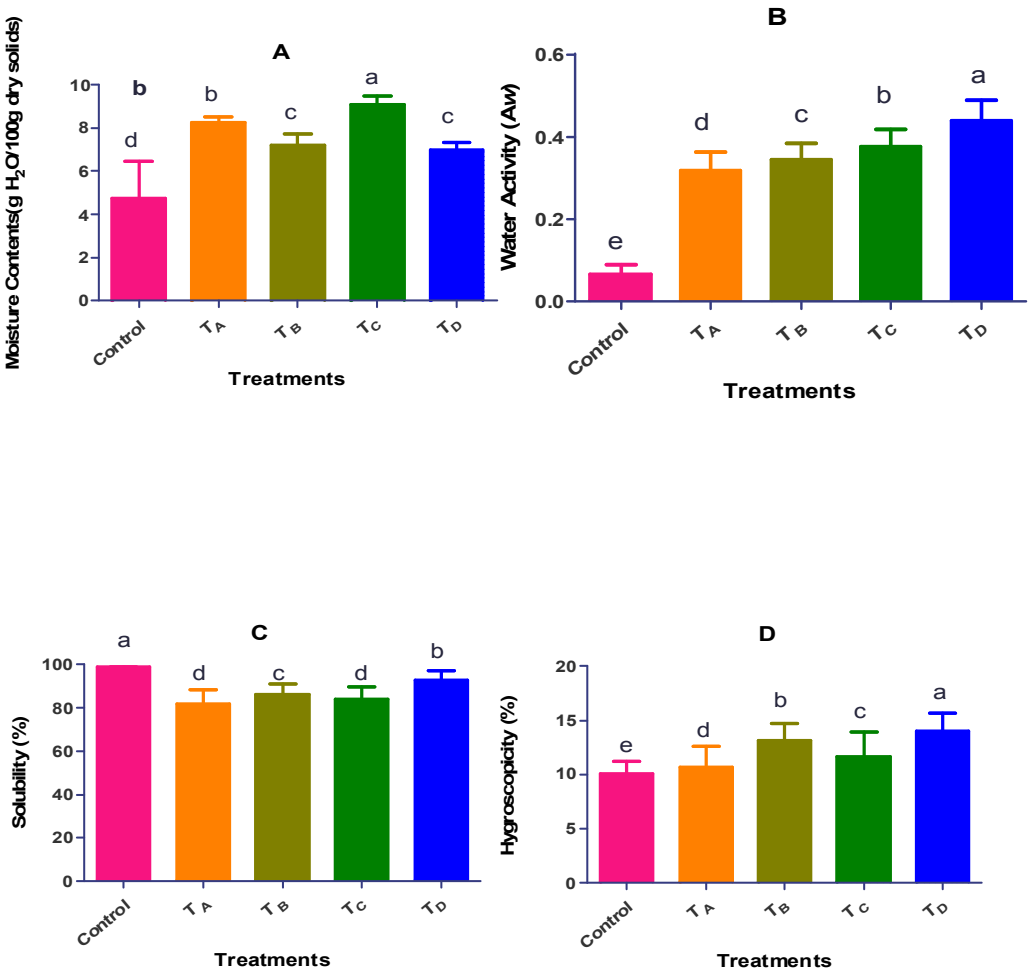
hygroscopic values, regardless the presence of higher contents of moisture [25]. The aforesaid behavior was also reported by Khazaei, et al. [31].The lower values of hygroscopicity for the all lyophilized products mainly attributed to the bigger particle size, since the bigger the particle size, the lesser the uncovered surface area, therefore low down the water absorption [26, 58].

The stability of microencapsulated powdered products for the period of storage was principally determined by glass transition temperatures (T_g), the lower the T_g resulting in lower the stability of final product and vice versa. The glass transition temperatures (T_g) of all lyophilized products were of 15.86 to 45.0°C in range (Figure 1E). Amongst all lyophilized microencapsulated products, the T_C represented the highest glass transition temperature (45.0°C), proving maximum stability. Furthermore, other treatments also showed significant values for T_g except T_D. The glass transition temperature has been influenced by diverse factors, including moisture contents, chemical configuration and molecular mass of subjected matter [59]. Adhikari et al., 2004 found the lower transition temperatures of fruit drinks/extract were mainly due to the existence of elevated quantity of low molecular weight organic acids and polysaccharides [60]. Additionally, integration of microencapsulating agents in extracts has much predisposed on glass transition temperatures (T_g) which varied according to molecular weight of encapsulating material; increase in molecular weight of wall material resulting the increase in final T_g of the product. The results of our current work were corroborated with earlier findings [61-63]. The lyophilized microencapsulated product obtained from treatment D (T_D) represented the lower T_g because of lower molecular weight of MD. Moreover, this behavior was not noticed in T_C (10%GA), T_A (10% GA and 5% GE) and T_B (10%GA and 5%MD) due to the existence of uppermost molecular weight of GA in the term of quality and quality of wall material.



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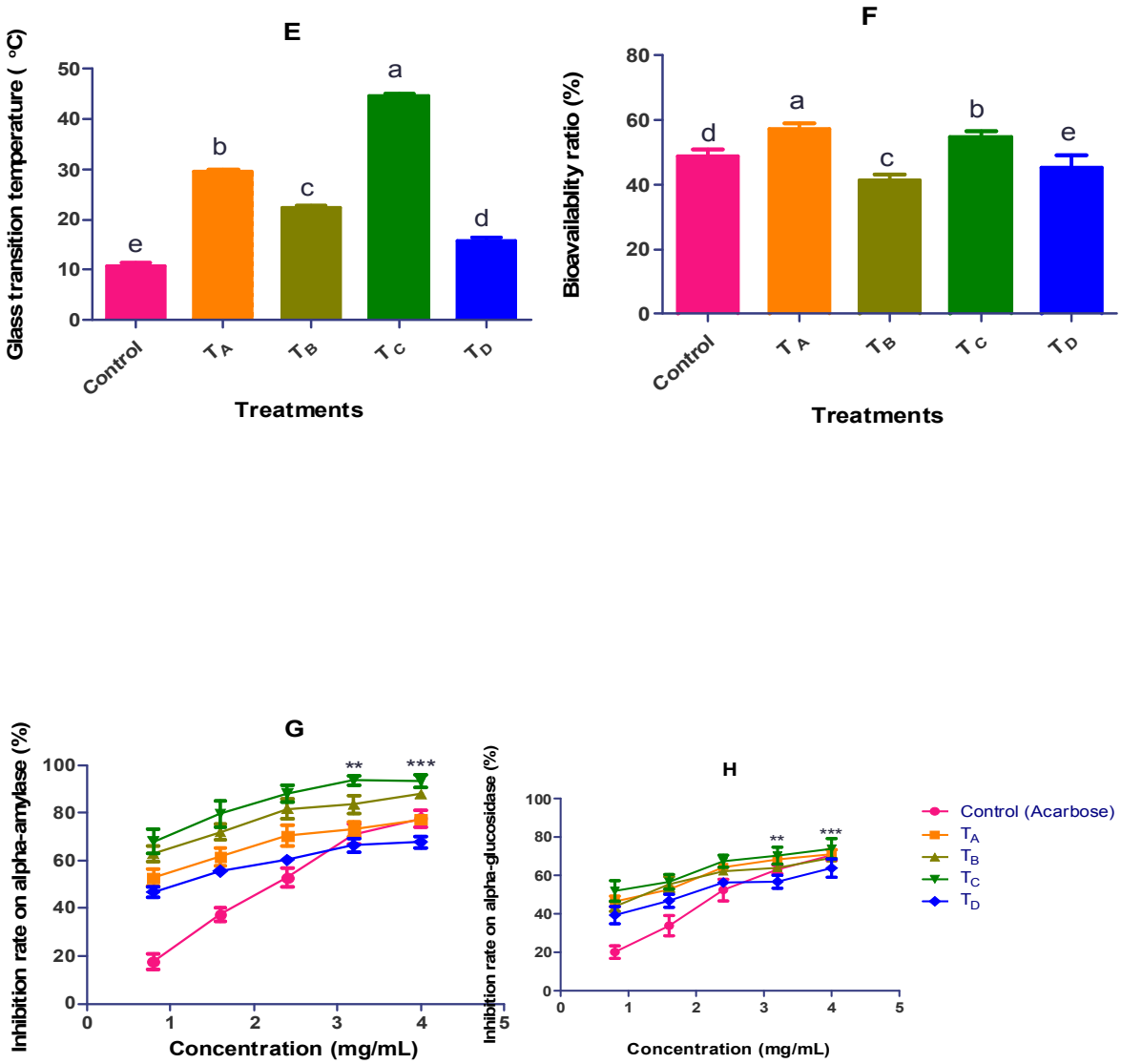

Figure 1(A-E): Physical properties of PHF extract microencapsulated with GA, GE, MD and their combinations by freeze-drying method.1F: bioavailability ratio (%), & 1G and IH: alpha-amylase and alpha-glucosidase inhibition activity (IC50). Treatment A (TA): Freeze-dried, with 5% GA and 5% GE; Treatment B (TB): Freeze-dried, with 5% GA and 5% MD; Treatment C (TC): Freeze-dried, with 10% GA; Treatment D (TD): Freeze-dried, with 10%.

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Table 1: Antioxidant components and antioxidant activities of PHF extracts microencapsulated with GA, GE, MD and their combinations by Freeze-drying method

Treatments	TPC *	TFC **	TCT ***	DPPH ****	[a]Beta-carotene	[b] ABTS
Control	26.72 ± 0.61[a]	6.848 ± 0.05 [a]	15.72 ± 0.3[a]	133.3 ± 1.79[a]	83.39 ± 0.79 [a]	3.687 ± 0.03 [a]
TA	22.89 ± 0.41[b]	2.76 ± 0.03[d]	9.383 ± .16[d]	74.73 ± 4.6[d,e]	64.71 ± 0.64[d,e]	3.197 ± 0.95[b]
TB	24.26 ± .085[a]	4.183 ± 0.07[b]	10.1 ± 0.13[c]	85.2 ± 0.5[b]	78.34 ± 0.51[b]	2.777 ± .125[c]
TC	25.26 ± 0.22[a]	5.233 ± 0.15[a]	12.46 ± 0.021[b]	78.11 ± 1.67[c]	75.4 ± 0.88[c]	2.733 ± 0.06[c]
TD	18.27 ± 0.15[c]	3.817 ± 0.03[c]	9.383 ± 0.07[d]	51.52 ± 0.72[f]	65.72 ± 0.92[d,e]	2.285 ± 0.072[d,e]

Note: Results displayed are a representation of triplicate quantifications per extract. Different letters within the same column indicate significant differences ($p < 0.05$) * Total phenolic contents (TPC) expressed as mg gallic acid equivalents (GAE) per g of dry extract; **Flavonoid content expressed as mg quercetin equivalents (QE) per g of dry extract; ***Total condensed tannin content based on calibration curve of (+)-catechin, expressed as mg catechin equivalents (CE) per g of dry extract. ****DPPH expressed as µmol/g sample on dry basis; [a]β-carotene of extracts (5 mg/mL) based on percent bleaching inhibition. [b] EC_{50} (mg/mL) is representative of the effective concentration at which 50% of $ABTS^{+}$ radicals were scavenged. The Dunnett's test was to evaluate the significance with confidence level was set to 95%. TA: Freeze-dried, with 5% GA and 5% GE; TB: Freeze-dried, with 5% GA and 5% MD; TC: Freeze-dried, with 10% GA; TD: Freeze-dried, with 10%.

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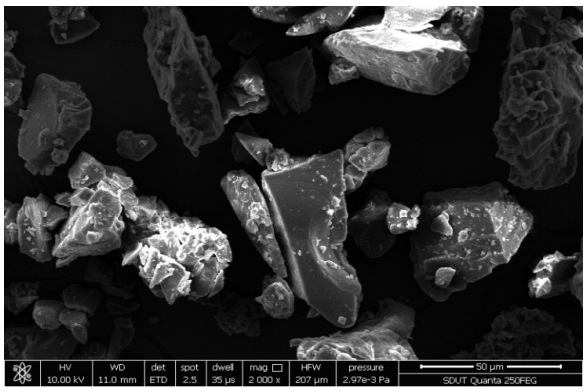
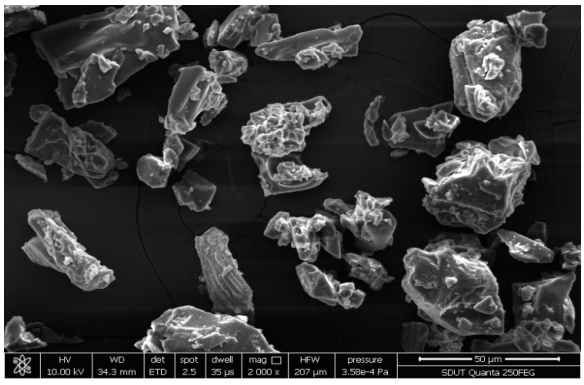
4.3. Size distribution and Morphology of Microencapsulated powders

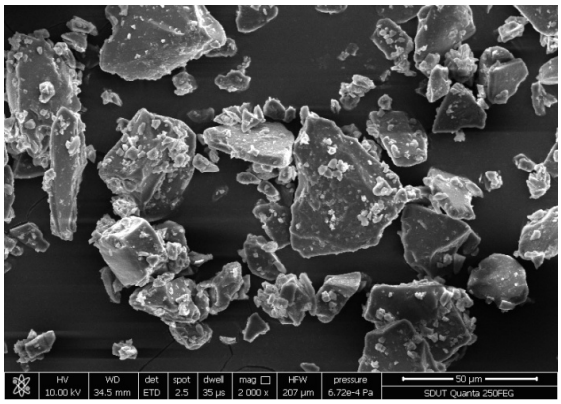
Different polymers exhibited particular protection capacity, so the evaluation of microencapsulated products is very crucial. This aforesaid capacity elaborated the extent of micro-pores and reliability of encapsulated micro-particles [64]. The structural analysis of the encapsulated products from the lyophilization methodology was conducted by the means of scanning electron microscope (Quanta 250 EFI). Comparison of the images illustrated the noticeable variation in term of particle structure and size allocation amongst the different microencapsulated products and their combination attained after lyophilization. Figure 2A, 2B, 2C, 2D demonstrated the morphology of all freeze-dried microencapsulated products. As can be seen all lyophilized products presented the irregular shape like broken glass with appreciable proportion of pores on surface. The outcome of current investigation has agreement with the recent work explored by Kuch and Norena [65]. These authors studied on morphological aspects of lyophilized products, made up from the peel of grapes and pomace of *Averrhoa carambola* and presented the final product as porous, uneven and brittle confirmation; furthermore they also described the reason behind the high porosity of lyophilized products as development of ice crystals had happen in material which as a result retarded the breakdown of final configuration and hence less change in volume occurred.



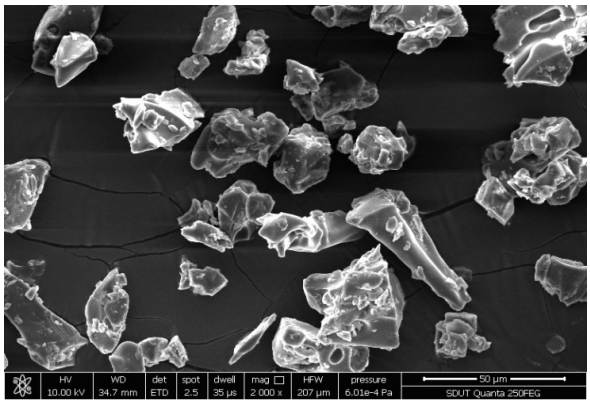
Treatment A (TA)



Treatment B (TB)



Treatment C (TC)



Treatment D (TD)

Figure 2: Micrographs of PHF extract microencapsulated with GA, GE, MD and their combinations by freeze-drying method.Treatment A (TA): Freeze-dried, with 5% GA and 5% GE; Treatment B (TB): Freeze-dried, with 5% GA and 5% MD; Treatment C (TC): Freeze-dried, with 10% GA; Treatment D (TD): Freeze-dried, with 10%.

There was a direct association between span value and dispersal of particle size, the lesser span value demonstrating a uniform distribution of micro-particles [66]. The size of micro-particles from the final products was in the range of 18.08 to 391.30 µm. T_A explored the higher particle size (more than 287µm), whereas T_D showed the lowest one. Our current work is consistent with prior investigation, examined by other authors [58] who found that the particle size of microencapsulated product via freeze-dying method reached up to 300µm. The bigger particle dimension of lyophilized products was mainly attributed to rapid freezing and less availability of force to crush the freeze drop during lyophilization [67, 68]. Moreover, particle size was also influenced by crushing procedure which was generally accustomed for size reduction after lyophilization.

4.4. Alpha-Amylase & Alpha-Glucosidase Inhibition

Type-II DM an outcome of insulin resistance is a metabolic disease that, according to the latest data for the World Health Organization in 2014, impinges on 9% of the world's population, both in developed and developing countries, and directly caused 1.5 million deaths in that single year [1, 2]. In order to hamper the side effects of type-II DM, insulin injection and usage of anti-hyperglycaemic substances are two key conventional approaches. The management of the blood sugar level is effective and novel approach to overcome the diabetes mellitus and related complications. Inhibitors of carbohydrate hydrolyzing enzymes (i.e.: α-amylase and α-glycosidase) have been practically valuable as oral hypoglycemic drugs and regarded as a reliable indicator for the efficacy of therapeutic agents [69-71]. Several α- amylase inhibitors including acarbose, miglitol and voglibose are clinically useful to treat diabetes but these are expensive and have considerable clinical side effects. Medicinal plants have great potential to retard the absorption of glucose by inhibiting the saccharides hydrolyzing enzymes [72-74].

There was an attempt to explore the remarkable drugs from medicinal plants featured with elevated potency and less adverse effects than existing drugs [75, 76]. Therefore, screening and isolation of inhibitors from plants for these enzymes are escalating.

In the aforementioned context, our microencapsulated polyphenolic enriched powders were investigated for α-amylase and α-glycosidase inhibition as shown in Figure 1G & 1H. Diverse classes of polyphenolic compounds in the current PHF extract were detected likewise: flavonoids, alkaloids, terpenoids, lignans, glycerophospholipid, prenol lipids and their derivatives (detailed in Section 2.5), which eventually may considered for antidiabetic potential of microencapsulated powders of current study. The treatment T_C (10% GA) demonstrated the highest inhibition at concentration of 4mg/mL, for alpha-amylase (93.33 ± 2.65, with IC_{50} value 1.47 mg/mL ± 0.57) and alpha-glucosidase (73.39 ± 1.66 with IC_{50} value 2.03 ± 0.45 mg/mL), representing highest antidiabetic potential. Previously, none of investigation has yet been carried out on lyophilized aforementioned microencapsulated PHF products. Additionally, there is no report on microencapsulation of polyphenol enriched extract from PHF and their characterization for anti-diabetic potential purposes, which eventually facilitate to take decision for commercialization of microencapsulated products *i.e.* polyphenols enriched nutrient supplement.

4.5. Bioavailable TP contents

TP contents present in dialyzable fraction of final products were illustrated in Table 2 (mean value ± standard deviation). TP bioavailability ratios, articulated in the term of percentage, were computed by using the equation as followed:

$$Bav(\%) = \frac{[\text{TP}]\text{Dialyzable}}{[\text{TP}]\ \text{Total}} \times 100$$

Where, B*av* (%) represented the percentage (%) for TP bioavailability, whereas [TP] Total and [TP] Dialyzable demonstrated TP concentrations after the PLE extraction method and in vitro digestion procedure respectively.

Table 2.represented the Dialyzable TPs, were in the range of 8.21–13.32mg GAE/g. Figure 1F depicted the bioavailability ratio (%) for all freeze-dried microencapsulated products. Treatment T_A

and T_C demonstrated the excellent bioavailability ratios (%) i.e. 57.25 and 54.64 % respectively, there was no significant difference in T_B and T_D. Furthermore No research has yet been conducted on in vitro dialyzability analysis of aforesaid microencapsulated PHF products.

Table 2: Average diameter and particle size distribution (Span) of the PHF extract microencapsulated with GA, GE, MD and their combinations by freeze-drying method.

Treatments	Average Diameter (μm)	Span
TA	151.13	1.74
TB	76.15	1.21
TC	92.79	2.88
TD	18.95	1.52

Treatment A (c; Treatment B (TB): Freeze-dried, with 5% GA and 5% MD; Treatment C (TC): Freeze-dried, with 10% GA; Treatment D (TD): Freeze-dried, with 10%.

4.6. Acute toxicity

No toxic effects and mortality were observed at a dose of 2000 mg/kg by gavage. Consequently, microencapsulated products of PHF extract were regarded as safe for consumption.

4.7. Bioactive Compounds from LC-ESI-QTOF-MS analysis

The ethanolic extract of freeze dried fine powder of PHF was a multifaceted mixture of compounds. Figure 3 characterized the chromatogram of said ethanolic extract. The bioactive compounds were recognized by means of the comparing retention times (RT) and MS/MS spectra granted by QTOF-MS with those of valid standards wherever obtainable and via elucidation of MS and MS/MS spectra from QTOF-MS merged with data available in literature. MS data of identified compounds has been recapitulated in the Table 3 including calculated *m/z* for molecular formulas provided, main fragment obtained by MS/MS, error and proposed compound for each peak. Diverse classes of polyphenolic compounds have been discovered in the ethanolic extract of PHF. The major detected classes of these polyphenolic bioactive compunds are flavonoids, alkaloids, terpenoids, lignans, glycerophospholipid and prenol lipids.

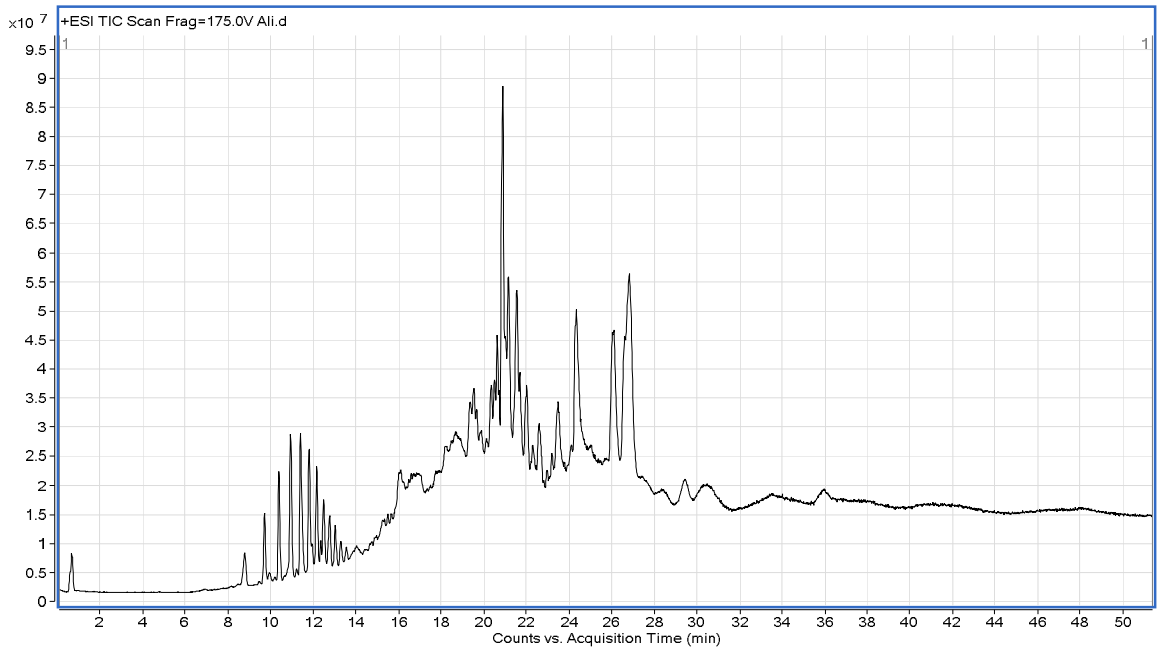


Figure 3: Chromatogram of the Ethanolic extract derived from freeze dried powder of PHF

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Table 3: Bioactive Compounds identified in Ethanolic Extract of PHF

Peak No.	RT (min)	Assigned Compound Name	Elemental Composition	m/z [M+H] +	Difference (mDa)
1	8.773	Caffeic acid 4-sulfate	C9H8O7S	261.128	0.71
2	9.458	Steviol	C20H30O3	319.1329	-0.92
3	9.708	Antherospermidine	C18H11NO4	305.1541	0.15
4	9.904	ERIODICTYOL	C15H12O6	289.1231	-0.14
5	9.95	Phloretin	C15H14O5	275.1077	1.04
6	10.177	Zanthobisquinolone	C21H18N2O4	363.1589	0.57
7	10.384	Murrayazolinol	C23H25NO2	349.1795	0.72
8	10.639	Patuletin	C16H12O8	333.1488	0.34
9	10.746	Albanin d	C25H26O5	407.1849	0.84
10	10.834	3,5,8,3',4',5'-Hexahydroxyflavone	C15H10O8	319.1329	0.31
11	10.918	Myricetin	C15H10O8	319.1692	0.3
12	10.936	Dehydroneotenone	C19H12O6	393.2055	0.77
13	11.219	Carissanol	C20H24O7	377.1746	0.86
14	11.4	Epigallocatechin 3-O-cinnamate	C24H20O8	437.2313	-0.13
15	11.479	Catalpol	C15H22O10	363.195	0.8
16	11.528	Secoisolariciresinol	C20H26O6	363.1589	0.46
17	11.805	Quercetagetin 7-glucoside	C21H20O13	481.2572	-0.02
18	11.945	Cajaflavanone	C25H26O5	407.2208	-0.17
19	12.163	Barbatoflavan	C24H28O13	525.2828	0.9
20	12.348	Celastrol	C29H38O4	451.2469	0.04
21	12.484	6-Gingerol	C17H26O4	296.1487	-1.92
22	12.77	Euphorbia diterpenoid 3	C33H40O11	613.3348	0.89
23	13.017	2-Hexaprenyl-6-methoxyphenol	C37H56O2	534.3435	0.67
24	13.278	PE(P-16:0/18:2(9Z,12Z))	C39H74NO7P	701.3867	-1.1
25	13.569	Buddliedin A	C17H24O3	277.1385	-0.24

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26	16.104	Phytosphingosine	C18H39NO3	318.2974	-0.04
27	17.059	1-EICOSENE	C20H40	282.2015	0.84
28	19.53	Tectorigenin	C16H12O6	301.1391	0.18
29	20.643	3-O-cis-Coumaroylmaslinic acid	C39H54O6	619.3973	-0.4
30	20.884	PA(18:3(6Z,9Z,12Z)/20:3(8Z,11Z,14Z))	C41H69O8P	721.4644	0.32
31	21.141	Eurysterol A sulfonic acid	C27H46O7S	515.3518	0.33
32	21.548	Citflavanone	C20H18O5	338.3391	1.25
33	21.698	Mesaconitine	C33H45NO11	631.4345	-0.17
34	22.01	Xanthoangelol	C25H28O4	393.294	0.44
35	22.385	PA(15:0/22:4(7Z,10Z,13Z,16Z))	C40H71O8P	711.4757	0.56
36	22.589	2',5,6-trimethoxyflavone	C18H16O5	312.3236	1.42
37	22.613	Ubiquinol-8	C49H76O4	729.5073	0.38
38	22.646	Epicalyxin J	C42H38O9	686.4852	0.14
39	22.967	Luteone	C20H18O6	354.37	1.64
40	23.177	Luteolin 4'-sulfate	C15H10O9S	366.3702	1.43
41	23.524	Quercetin 3-(6''-malonylglucoside)-7-glucoside	C30H32O20	713.5121	0.64
42	24.348	Phytoene	C40H64	545.1143	0.39
43	26.083	Epigallocatechin 3,3',-di-O-gallate	C29H22O15	610.1796	0.62
44	26.819	Kaempferol 3-(2'',3''-diacetyl-4''-p-coumaroylrhamnoside)	C34H30O14	663.4496	0.47
45	29.446	Delphinidin 3-(6''-malonyl-glucoside)	C24H23O15	684.1982	0.21

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A total of 17th compounds were regarded as Flavanoids and their derivatives. These compounds have been reported priory to possess anti-inflammatory, antinociceptive and antioxidative activities, such as to inhibit lipid peroxidation, chelate redox-active metals and inhibit free-radical mediated events and even increasing resistance to DNA strand breakage [77]. The brief description of each bioactive compounds are elaborated;Eriodictyol, *m/z* 289.1231 *[M+H]*$^+$) (4th detected compound)is a flavonoid and further classified as flavanones. These are compounds containing a flavan-3-one moiety, having a structure distinguished by a 2-phenyl-3, 4-dihydro-2H-1-benzopyran bearing a ketone at the carbon C3 [78] have previously proven pro and antioxidant activities [79]. The compound 8th (*i.e.* Patuletin *m/z* 333.1488 *[M+H]*), compound 10th (*i.e.* 3, 5, 8, 3', 4', 5'-Hexahydroxyflavone, *m/z* 319.1329 *[M+H]*) and compound 40th (*i.e.* Luteolin 4'-sulfate, *m/z* 366.3702 *[M+H]*) demonstrated the class flavonols. These are compounds that contain a flavone (2-phenyl-1-benzopyran-4-one) backbone carrying a hydroxyl group at the 3-position. These compounds were excessively present in coffee beans, herbs and spices, and pulses. Myricetin, *m/z* 319.1692 *[M+H]* (11th detected compound) is a natural flavonoid present in plant kingdom including berries, grapes, vegetables and variety of herbs, walnuts was also considered as rich dietary source of myricetin. This bioactive flavonoid has previous proven anti-inflammatory and antioxidant activity [80]. The compound (19th) Barbatoflavan belongs to flavonols *m/z* 525.2828 *[M+H]*, demonstrated scavenging properties towards the DPPH radicals [81]. The 32th peak is of Citflavanone, *m/z* 338.3391 *[M+H]*, belong to flavonoids class having anti-dengi, anti- malarial activities [82, 83].

Moreover, the Compound 9th (*i.e.* Albanin d, *m/z* 407.1849 *[M+H))*, compound 18th (*i.e.* Cajaflavanone, *m/z* 407.2208 *[M+H]*) and compound 39th (*i.e.* luteone *m/z* 354.37 *[M+H]*), belongs to a class typically known as 6-prenylated flavones. 6-prenylated flavones are flavones that features a C5-isoprenoid substituent at the 6-position. These bioactive compounds are insoluble in aqueous solution and designated as a faintly acidic compound. These compounds previously discovered in fruits, peas and pulses and considered to be flavonoid lipid molecule. Other detected flavonoid lipid molecule was the 2', 5, 6-trimethoxyflavone, *m/z* 312.3236 *[M+H]* (Peak 36) which are related to class named as 6-o-methylated flavonoids. These bioactive flavonoids compounds had a structure contain methoxy groups attached to the C6 atom of the flavonoid backbone. Tectorigenin (*i.e* compound 28th, *m/z* 301.1391 *[M+H]*) is an O-methylated isoflavone, Isoflavones are polycyclic compounds containing a 2-isoflavene skeleton which bears a ketone group at the C4 carbon atom. The 12th detected compound (*i.e.* dehydroneotenone, *m/z* 3393.2055 *[M+ H])* also recognized as Isoflavones. Epigallocatechin 3-O-cinnamate, *m/z* 437.2313 *[M+H])* (Peak 14th) is a flavan-3-ol comprising a benzopyran-3,5,7-triol attached with a 3,4,5-hydroxyphenyl moiety and Epigallocatechin 3,3',-di-O-gallate, *m/z* 610.1796 *[M+H]*, belongs to class catechin gallates, containing a gallate moiety glycosidically linked to a catechin,thus, (-)-epigallocatechin 3,3'-di-gallate is also considered to be a flavonoid lipid molecule.

In addition to aforementioned flavonoids, there was also detection of some other bioactive compounds, which belong to sub class flavonoids glycosides: Quercetagetin 7-glucoside (Compound 17th, *m/z* 481.2572 *[M+H])* and Quercetin 3-(6''-malonylglucoside)-7-glucoside (Compound 41th,*m/z* 713.5121 *[M+H])* wererecognized as flavonoid-7-o-glycosides. These are phenolic compounds containing a flavonoid moiety which is O-glycosidically linked to carbohydrate moiety at the C7-position. These derivatives of flavonoids have priory proved strong antioxidant activity [84-85].

The 44th detected compound (Kaempferol 3-(2'', 3''-diacetyl-4''-p-coumaroylrhamnoside), *m/z* 663.4496 *[M+H])* and 45th detected compound (Delphinidin 3-(6''-malonyl-glucoside), *m/z* 684.1982*[M+H]*) were generally categorized as flavonoid-3-o-glycosides. These bioactive compounds contained a structure in which flavonoid moiety is O-glycosidically attached at the C3-position with carbohydrate moiety. Catalpol *m/z* 363.195 *[M+H]* (Compound 15th) is a iridoid glucoside and has been found to be present in large quantities in the root of *Romania glutinosa.* As a traditional medicine, catalpol demonstrates a variety of biological activities including anticancer, neuro-protective, anti-inflammatory, diuretic, hypoglycemic and anti-hepatitis virus effects. Previous studies have also provided some clues that catalpol can affect energy metabolism through increasing mitochondrial

biogenesis, enhancing endogenous antioxidant enzymatic activities and inhibiting free radical generation ultimately attenuates oxidative stress [86].

The peak 33th (*i.e.* Mesaconitine, *m/z* 631.4345 *[M+H]*) and peak 3rd (*i.e.* Antherospermidine, *m/z* 305.1541 *[M+H]* +) were the member of group named alkaloids, later have a structure that contains an aminoethylphenanthrene moiety. Atherosperminine has been cited to be in fruits and bark of *Cryptocarya nigra* (Lauraceae) and have strong antioxidant, antimlarial and antimicrobial activities [68]. Steviol, *m/z* 31 9.1329 *[M+H]* + designated as compound 2nd in our list of metabolites, is diterpene alkaloids with a structure that is based on the kaurane skeleton. It possesses a [3, 2, 1]-bicyclic ring system with C15-C16 bridge connected to C13, forming the five-membered ring D. This compound was excessively found in different sorts of fruits and primarily responsible for the sweet taste of stevia leaves. This compound is considered safe for human consumption and was approved as a food additive by the Food and Drugs Administration (FDA) and European Food Safety Authority (EFSA) it helps to reduce the oxidative stress [88]. In addition, the peak 6th is of Zanthobisquinolone, *m/z* 363.1589 *[M+H]* and peak 7th is of Murrayazolinol, *m/z* 349.1795 *[M+H]* belongs to the class Quinolines and their derivatives, also alkaloid in nature. These are usually present in herbs, spices and some fruits [89-91].Various anti-malarial, antiparasitic, antibacterial and antiviral drugs do contain a major constitute of aforementioned bioactive compound [92].

Besides flavonoid and alkaloids, these are also some compounds which have appreciable share, belong to class prenol lipids. Likewise; the 20thdetected compounds named as Celastrol, (plant-derived triterpene) *m/z* 451.2469 *[M+H]*, have previously proven antioxidant and anti-inflammatory activity and preventedthe neuronal degeneration in Alzheimer's disease (AD) [93]. The 23th compound designated as 2-Hexaprenyl-6 methoxyphenol, *m/z* 534.3435 *[M+H]* is involving in the ubiquinone biosynthesis pathway. It is formed from 3-Hexaprenyl-4-hydroxy-5-methoxybenzoate. In the venture of ubiquinone biosynthesis monooxygenase Coq6T, the aforementioned compound (23th) is eventually converted into 2-Hexaprenyl-6-methoxy-1, 4-benzoquinone**.** Ubiquinol 8, *m/z* 729.5073 *[M+H]* (compound 37th) belongs to organic compounds known as polyprenyl quinols. These are compounds encompassing a poly-isoprene chain attached to a quinol (hydroquinone) at the second ring position. It is the reduced configuration of ubiquinone-8. It plays a function as an electron transporter in mitochondrial membrane, where it carries two electrons from either complex I (*i.e.* NADH dehydrogenase) or complex II (*i.e.* succinate-ubiquinone reductase) to complex III. Phytoene, *m/z* 545.1143*[M+H]* (compound 42th) is member of class regarded as carotenes and further belongs to family carotenoids. These are unsaturated hydrocarbons comprising of eight repeated isoprene units. They have also previously proven antioxidant, anticancer activity and facilitate to reduce the complications [94].

Amongst the known natural bioactive compounds in nature, terpenoids are considered to be of approximately 60%. Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies, for instance; *Euphorbia* diterpenoids 3 (Compound 22th, *m/z* 613.3348 *[M+H]*) possesses a variety of different core frameworks and exhibit a diverse array of beneficial activities, including anti-tumor, anti-inflammation, and immune-modulatory features, which was regarded as excellent source in term of scientifically attraction [90, 91]. Buddledin A (Compound 25th, *m/z* 277.1385 *[M+H]*, a sesquiterpenoid based on a humulane skeleton (a novel terpenes from *Buddleja globosa*) displaying selective antifungal activity against dermatophytes [95] in the same manner, 3-O-cis-Coumaroylmaslinic acid (Compound 29th (Triterpenoids), *m/z* 619.3973 *[M+H]*), it is normally present in fruits herbs and spices, and have ability to attenuate oxidative stress.

Lignans were usually found in fruits and have proved strong anticancer and antioxidant activities. Among them, Secoisolariciresinol, *m/z* 363.1589 *[M+H]* (Compound 16th) belongs to class dibenzylbutane lignans, containing a 2, 3-dibenzylbutane-1, 4-diol moiety. It was present in a number of food items such as American butterfish, brazil nut, fireweed, and oriental wheat [96]. Carissanol, *m/z* 319.1692 *[M+H]* (Compound 13th) on the other hand belongs to the class furanoid lignans containing a 3, 4-dibenzyloxolan-2-ol moiety [97].

Phloretin (peak 5th, *m/z* 275.1077 *[M+H]*) and Xanthoangelol (peak 34th, *m/z* 393.294 *[M+H]*) belong s to class chalcones and dihydrochalcones. Phloretin was previously reported to promote

potent anti-oxidative activities in peroxynitrite scavenging and the inhibition of lipid peroxidation. It is present in apples, pears and tomatoes, has been found to inhibit the growth of several cancer cells [98], whereas Xanthoangelol (obligate intermediated in flavonoid biosynthesis) have antitumor and anti-metastatic features [99].

Other detected compounds which were not discussed in detailed such as compound 24th, 30th, 35th, 26thare intermediate products of either metabolism or biosynthesis of amino acids, (phosphor or/and sphingo) lipids. For instance, metabolite 24th represented a phosphatidylethanolamine, is an anchor protein, produced as an intermediate in Glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway,while30th compound *m/z* 721.4644 *[M+H]* and compound 35th, *m/z* 711.4757 *[M+H]* are the phosphatidic acids, produced in glycerolipid biosynthesis. The existences of such compounds are mainly attributed to the seeds of *Hygrophila spinosa* T. Anders [100]. The 26th peak recognized as Phytosphingosine, *m/z* 318.2974 *[M+H]*, is an intermediate compound synthesized between dihrdro-shingosine and phyto-ceramide in shingophospholipid metabolism. Phospholipids have diverse functions in varied processes of cell *i.e.* and apoptosis, cell propagation, cell to cell interaction, differentiation. Furthermore, phytosphingosine is naturally occurring sphingoid bases, fungi and plants are the rich source of phytosphingosine. It is structurally similar to sphingosine; phytosphingosine possesses a hydroxyl group at C-4 of the sphingoid long-chain base. phytosphingosine induces apoptotic cell death in human cancer cells by direct activation of caspase 8, and by mitochondrial translocation of Bax and subsequent release of cytochrome C into cytoplasm, providing a potential mechanism for the anticancer activity of phytosphingosine [101].

Peak 21th showed the molecular formula $C_{17}H_{26}O_4$ and its MS spectrum presented a fragmentation at *m/z* 296.1487 $[M+H]^+$, which correspond to a compound of phenols class, it is the active constituent of fresh ginger. Chemically, gingerol is a relative of capsaicin, belongs to the class of organic compounds known as gingerols (phenols). These are compounds containing a gingerol moiety, which is structurally characterized by a 4-hydroxy-3-methoxyphenyl group substituted at the C6 carbon atom by a 5-hydroxy-alkane-3-one [102].

The metabolite 01, *m/z* 261.128 $[M+H]^+$ have been referred to Caffeic acid 4-sulfate (polyphenol) belongs to a class Cinnamic acids and their derivatives. Hydroxycinnamic acids are compounds containing a cinnamic acid where the benzene ring is hydroxylated. It is one of the most representative phenolic acids in fruits and vegetables which haveexcellent antioxidative potentialand anti-carcinogenic activity [90].

The peak 27th, Eicosene, belongs to a class regarded as unsaturated aliphatic hydrocarbons. These are aliphatic hydrocarbons that have one or more double/triple bonds. 10-eicosene can be found in herbs and spices; 10-Eicosene is usually present in herbs and spices and has good antioxidant and antimicrobial activity [91, 92].

The 31th compound is of Eurysterol A sulfonic acid, *m/z* 515.3518 *[M+H]*, A steroid sulfate that is 5α-cholestane substituted by hydroxy groups at positions 5 and 6, a bridged oxolane at positions 8 and 19 pand a sulfate group at position 3. It has proven cytotoxic and antifungal activities [91].
Epicalyxin J, *m/z* 686.4852 *[M+H]* (Compound 38th) is the diarylheptanoid are a relatively small class of plant secondary metabolites. Diarylheptanoids consist of two aromatic rings (aryl groups) A diarylheptanoid is an intermediate in the biosynthesis of phenylphenalenones in *Anigozanthos preissii* it is strong potential against human fibro-sarcoma cells [103].

As can been concluded that the current PHF is the mixture of previously proven [31-34] health promoting herbs' parts, so diversity and abundance of such detected antioxidant substances/metabolites not only made sense but also verify the outcomes. Taking together, this is the first study which exploited the metabolite profiling of said PHF enriched with antioxidants and their evaluation for bioavailability and anti-diabetic potential after encapsulation.

5. Conclusions

In the current study, PHF polyphenolic extract was microencapsulated by utilizing GA, GE, and MD as encapsulating wall materials, resulting powdered products had withholding capacity of TPC more than 85% except T_D (68.22%), while TFC and TCT were found near to 60% except T_A.

Furthermore, elevated antioxidant activity was also revealed for T_B and T_C and reasonable for T_A and T_D, representing noteworthy and positive correlation of antioxidant assays to all aforementioned antioxidant components. Taking all results into consideration, T_B (5% GA and 5% MD) and T_C (10% GA) showed the best performance attributable to the superior preservation of antioxidant components and antioxidant activity by means of DPPH and β-carotene assays and significant for an $ABTS^+$ radical scavenging activity, augmented by low contents of moisture, water activity (*aw*), particle dimension and elevated solubility, hygroscopicity and T_g. Additionally, the aforementioned treatments also demonstrated the excellent morphological features with asymmetrical (irregular) micro-particle structures, depicted lower prevalence of coarseness and crankiness. Moreover, T_B, T_C and T_A also characterized the highest antidiabetic potential by reason of their significant inhibition rate for alpha-amylase and alpha-glucosidase. In the context of bioavailability, T_B and T_C also demonstrated the excellent bioavailability ratios (%) (*i.e.* more than 50% and 40% respectively). In addition, no mice proved any toxicity sign at a dose of 2000 mg/kg by gavage for any treatment. In the conclusive manner, we recommended the T_B and T_C as result of their incredible potential for its usage in nutraceutical and functional products while masking the undesirable flavor distinctiveness of herbs/herbal extracts.

Conflict of Interest:Authors declare no competing interests.

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