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Phytochemical analysis and *in vitro* antioxidant activity of *Solanum tuberosum* peel using high-resolution liquid chromatography mass spectrometry (hr-lcms) and ftir

Análisis fitoquímico y actividad antioxidante in vitro de la cáscara de *Solanum tuberosum* mediante espectrometría de masas por cromatografía líquida de alta resolución (hr-lcms) y ftir

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ABSTRACT

Potato peel (*PP*) is high in bioactive compounds and has an immense important in the nutraceutical sector, a wide spectrum of bioactive secondary metabolites produced by potato peel can be used to build novel medications and functional foods. It has been demonstrated that complex bioactive chemicals must be analyzed using analytical methods such as HR-LCMS. The current project's goal was to study the bioactive metabolites found in the methanolic extract of potato peel (*MEPP*) using total phenolic (TPC), flavanoids (TFC), *in vitro* antioxidant activity, HR-LCMS and FTIR. Using Q-TOF LC/MS and Agilent MassHunter Profiler software to investigate the bioactive components of *PP*, researchers discovered 28 bioactive chemicals such as 4,7-Dihydroxy-2H-1-benzopyran-2-one.4-Coumaroyl-2-hydroxyputrescine,6,8dihydroxypurine,6'-methoxypolygoacetophenoside, Alloxanthin, Butyl 3-O-caffeoylquinate, Chlorogenic acid, Cularicine, Feruloylputrescine, Feruloylput, Quinic acid, Gerberinol, Grossamide, Quercetagetin that have anticancer, antimicrobial, anti-inflammatory, and antitumor activity. The current findings of this study reveal the presence of significant phytocompounds in PP and are valuable for future research into developing functional foods from PP to avoid a variety of diseases. Current study is aimed at discovering all-natural treatments for a wide range of ailments and conditions.

Keywords: Potato peel, HR-LCMS, FTIR, Bioactive compounds, TPC, TFC

RESUMEN

La cáscara de papa (PP) es rica en compuestos bioactivos y tiene una inmensa importancia en el sector nutracéutico. Un amplio espectro de metabolitos secundarios bioactivos producidos por la cáscara de papa se puede

utilizar para construir nuevos medicamentos y alimentos funcionales. Se ha demostrado que las sustancias químicas bioactivas complejas deben analizarse mediante métodos analíticos como HR-LCMS. El objetivo del proyecto actual era estudiar los metabolitos bioactivos encontrados en el extracto metanólico de la cáscara de la papa (MEPP) utilizando fenólicos totales (TPC), flavonoides (TFC), actividad antioxidante in vitro, HR-LCMS y FTIR. Utilizando LC/MS Q-TOF y el software Agilent MassHunter Profiler para investigar los componentes bioactivos del PP. Los investigadores descubrieron 28 sustancias químicas bioactivas como 4,7-Dihidroxi-2H-1-benzopiran-2-ona. 4-Cumaroil-2-hidroxi putrescina, 6,8dihidroxipurina, 6'-metoxipoligoacetofenósido, aloxantina, butil 3-O-cafeoilquinato, ácido clorogénico, cularicina, feruloilputrescina, feruloilput, ácido quínico, gerberinol, grosamida y quercetagetina, que tienen actividad anticancerígena, antimicrobiana, antiinflamatoria y antitumoral. Los resultados actuales de este estudio revelan la presencia de importantes fitocompuestos en el PP y son valiosos para futuras investigaciones sobre el desarrollo de alimentos funcionales para evitar diversas enfermedades. Este estudio tiene como objetivo descubrir tratamientos totalmente naturales para una amplia gama de dolencias y afecciones.

Palabras clave: cáscara de papa, HR-LCMS, FTIR, compuestos bioactivos, TPC, TFC

INTRODUCTION

Solanum tuberosum, also known as potato, despite being a concentrated source of pharmacologically essential substances including minerals, dietary fibers, phenolics, and anthocyanins, they are unrestricted by technological intervention. Potato peels have high moisture content, attached starch, a fibrous texture, a dark color, and a musty flavor, which are the main sensory limits for successful use and storage (Joshi *et al.,* 2020). Utilizing potato peels' potential bioactive is necessary to fully exploit their nutritional value.

As a byproduct, the potato processing industries generate a large amount of potato peels, which have little or no value. These by-products are typically thrown in landfills, resulting in a severe impact on the environment, or used as low-value animal feed (Schieber *et al.,* 2009). Food processors, government agencies, and downstream processors are all aware of the business possibilities of high-volume by-products like potato peels. However, challenges remain in determining the best way to maximize benefit while minimizing cost and time of utilization. Potato peels are high in steroidal alkaloids (Slanina, 1990). The therapeutic value of potato peel has been established due to the presence of phenolic, polyphenolic compounds, anthocyanins, non-anthocyanin flavonoids, and glycoalkaloids, which are healthy compounds with antibacterial and antioxidant activities (Silva *et al.,* 2017).

Steroidal alkaloids, that possess a triterpenoid structure, are thought to be potential precursors for the manufacture of hormones, antibiotics, and anticancer medicines. Untapped sources of these chemicals include potato peels (Martinez *et al.,* 2021). The anticancer characteristics of solanum steroidal alkaloids have been

extensively researched; their necrotic mechanism of action against malignant cells renders them unsuitable for usage as an anticancer medication (Kenny *et al.,* 2013).

The conventional methods for characterizing bioactive chemicals include extraction, analysis, chromatographic separation, and spectroscopic identification. To discover the functionally effective bio-actives and make it easier to comprehend their impact on the target, it is essential to decipher the intricate chemistry of bioactive crude extracts utilizing high throughput and high-resolution techniques (Marulasiddaswamy *et al*. 2021). Using HRLC-MS and FTIR analysis, the current study attempted to examine the phytoconstituents present in *PP*.

MATERIALS AND METHODS

Chemicals: All the chemicals and reagents used for the research were of analytical grade and were procured from the reputed manufacturers.

Raw material collection and Preparation of extract: Potato Peel (*PP*) were air-dried at room temperature and pulverized into powder for extraction. The powder (100 g) was extracted by Microwave extractor (*Make Microsynth*) at 750 W in methanol (1:10 Solid-Solvent ratio) at 60° C and for 20 min. The mixture *MEPP* was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure to get an orange-yellow semi-solid extract, then the extract was stored at 4 degree C till further analysis.

Determination of Total Phenolics Content in MEPP: The Folin Ciocalteu reagent (FC Reagent) was used to calculate the total phenolics content. The standard for the calibration curve was gallic acid. The total phenolic content was expressed as gallic acid equivalents (mg/g),(Chun et al., 2003).

Determination of Total Flavonoids Content in MEPP: The total flavonoid concentration was assessed using a colorimetric assay. The standard for the calibration curve was Qurecitin. Per grams of material, the total flavonoids in the extract were estimated as mg/g of Qurecitin equivalents (Zhishen et al., 1999).

Determination of free radical scavenging activity: The total antioxidant activity of MEPP was measured in terms of the percentage of radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazil. A DPPH solution (1 mg/mL) was made by dissolving DPPH in methanol. The DPPH solution was diluted to 5 mL and the absorbance was calculated at 517 in a UV-Spectrophotometer (Gogavekar et al., 2012).

The following formula was used to determine the antioxidant activity.

$$\% \text{ Free Radical scavenging} = \frac{(\text{Absorbance of Control} - \text{Absorbance of sample})}{Absorbance\ of\ control}$$

HR-LCMS analysis of MEPP: G6550A MS Q-TOF was applied in a positive and negative mode with Dual AJS ESI Ion source. *Phenomenex Synergi* Polar-RP, 150 × 3 mm, 4 µm at 35 °C was used for the separation of phenolic compounds, and the flow rate was set at 0.300 mL/min. An aliquot of 5 µL from the extract was injected. Mobile phase (A) taken was 0.1% formic acid in water and mobile phase (B) taken was 95% acetonitrile with 0.1% formic acid. A full scan mode was reached in the range of 100–1000 amu and the conditions maintained were as following; capillary voltage (3500 V), nozzle voltage (1000 V), nitrogen gas flow rate (13 L/min) at 300°C and nebulization was set as 35 psig. Agilent Mass Hunter Work station Software (LC/MS Data Acquisition for 6200 series TOF/6500 series Q-TOF) was used for extraction and identification of phenolic compounds present in the potato peel extract.

Identification of polyphenols by LC–MS/MS analysis: The Identification of the chemical nature of extract was done by HRLC–MS/MS analysis; it was based upon the method described by (Akdeniz *et al*. 2018). The compounds obtained were listed and transferred automatically to MS/MS analysis in a further Q-TOF LC/MS analysis.

FTIR analysis of PP: Shimadzu spectrometers were used to study FTIR (IRAffinity 1S) , 2.0 mg of sample was combined with 200 mg of spectroscopic grade KBr before being compressed into a disc at 10 MPa for 3 minutes. The resulting spectra were collected with a resolution of 4 cm-1 in the 4000-400 cm^{-1} region. The obtained peaks and their associated functional groups were recognized. Peak values of the FTIR were recorded.

RESULT AND DISCUSSION

Total Phenolic, Total Flavanoid content and Antioxidant activity: The TPC result was 7.44±0.06 mg GAE/g. Every plant's phenolic content is intimately related to its antioxidant properties. Reducers, hydrogen donors, and free radical scavengers are all functions of phenolic compounds. TFC values were 7.83±0.07 mg QE/g, and the polarity of the extraction solvents affects the concentration of phenols and flavonoids (Jing *et al.,* 2015[a]), (Wojdyo *et al.,* 2007). The presence of polyphenols, flavonoids, and phenolic compounds may be associated to *MEPP*'s radical scavenging action, and phenolic compounds account for the majority of plant antioxidant activity (Jing *et al.,* 2015[b]), which is recorded as 63.13±0.06% (Illustrated in Table 1).

Table 1. TPC, TFC and in vitro antioxidant activity of Methanolic extract of PP

	TPC mg GAE/g	TFC mg QE/g	% Free radical scavenging activity
MEPP	7.44± 0.06	7.83± 0.07	63.13±0.06

(Results are mean ± SD of 3 determinations)

High Resolution-Liquid Chromatography-Mass spectrometry analysis (HR-LCMS) of MEPP: Chromatogram displays in Fig. 1 and 2 the approximate concentrations of several compounds that are present in *PP* and are eluted in accordance with the retention time and polarity. The height of the peak was used to calculate the relative concentration of the bioactive chemicals found in the plants. The molecules that were eluted at different times are examined by the mass spectrometer to ascertain the make-up and structure of the compounds. These mass spectra indicate the unique pattern of the molecule in the data base. The mobile phase was augmented with formic acid to optimize peak resolution. It was discovered that each resolved peak might represent many phytocompounds due to homology in polarity and chemical properties brought on by retaining the same retention time. Agilent iFunnel technology used electro spray to separate the contents, creating different ions fragments that were then concentrated using Agilent Jet Stream system for effective ion sampling and transmission. Table 1 lists the polarity, mass, and chemical formula for each molecule. Based on these values, 28 compounds were found in the methanolic extract of *PP* having phytochemical properties.

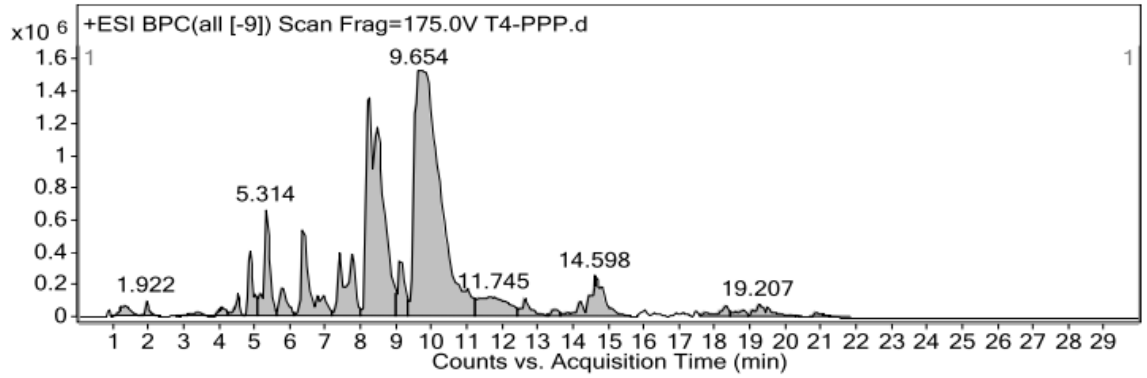

Figure 1 LC-MS analysis of MEPP at positive polarity.

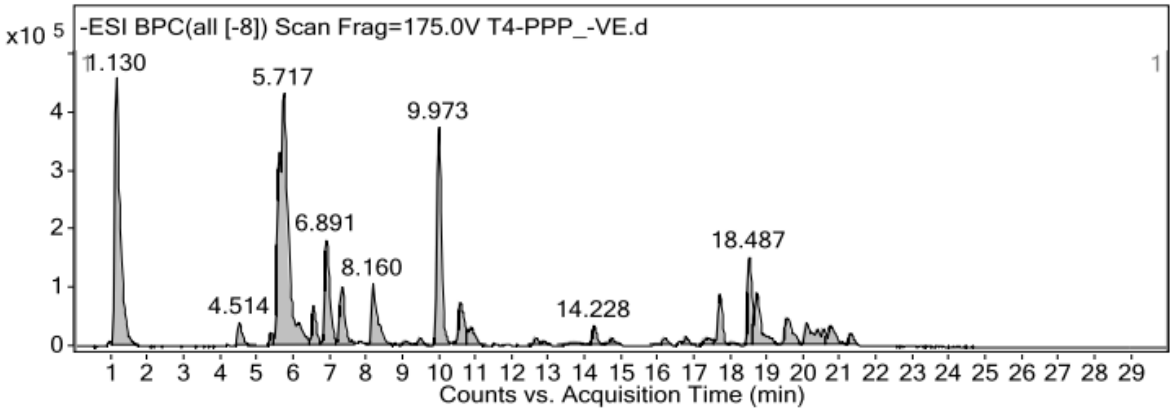

Figure 2 LC-MS analysis of MEPP at negative polarity

The primary compounds were predicted to belong to a variety of secondary metabolite categories based on the HR-LCMS investigation, including alkaloids, steroid, fatty acid, ester, phenol, hydroxycinnamic acid, Hydroxycoumarin, triterpenoids, polypeptides, glycoside, flavonoid, diterpenoid, and flavons. Fig. 3 depicts the various mass spectra of the discovered *PP* bioactives as shown in Table 2.

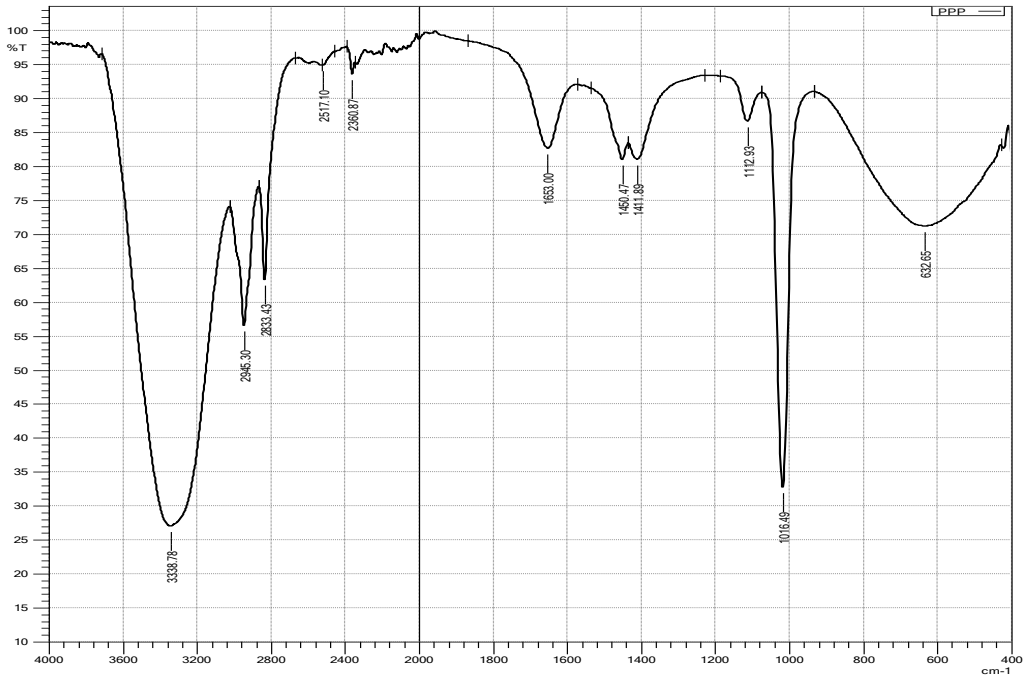

Figure 3. FTIR mass spectra of PP

Table 2. Chemical ingredients of Methanolic extract of PP by HRLC-MS

Sr.No	Compound Name	Formula Ion	Polarity	Mass	m/z	Compound nature
1	(3b,20R,22R)-3,20,27-Trihydroxy-1-oxowitha-5,24-dienolide 3-glucoside	$C_{34}H_{50}O_{11}$	Negative	634.342	693.3559	Triterpene Saponin
2	19-noretiocholanolone	$C_{18}H_{28}O_2$	Positive	276.2083	277.2151	Metabolite Of Nandrolone
3	4-(2-Nitroethyl)phenyl primeveroside	$C_{19}H_{27}NO_{12}$	Negative	461.1529	506.151	Phenolic Glycosides
4	4,7-Dihydroxy-2H-1-benzopyran-2-one	$C_9H_6O_4$	Negative	178.0282	177.021	7-Hydroxycoumarins
5	4-Coumaroyl-2-hydroxyputrescine	$C_{13}H_{18}N_2O_3$	Negative	250.1336	249.1263	Coumaric Acids And Derivatives.
6	6,8-dihydroxypurine	$C_5H_4N_4O_2$	Positive	152.0321	153.0392	Hypoxanthines
7	6'-methoxypolygoacetophenoside	$C_{15}H_{20}O_{10}$	Negative	360.1059	405.1042	Coumaric Acids And Derivatives
8	Alloxanthin	$C_{40}H_{52}O_2$	Negative	564.397	609.3978	Diterpenoid.

9	Asterosterol	$C2_6\ H_{42}\ O$	Positive	370.3166	371.3236	3-Hydroxy Steroid.
10	Butyl 3-O-caffeoylquinate	$C_{20}\ H_{26}\ O_9$	Positive	410.1574	433.1466	Quinic Acids and Derivatives
11	Chlorogenic acid	$C_{16}H_{18}O_9$	Negative	354.0944	357.15	Phenol
12	Cularicine	$C_{18}\ H_{17}\ N\ O_4$	Negative	311.1185	310.1116	Phenol
13	Diketospirilloxanthin/ 2,2'-diketospirilloxanthin	$C_{42}\ H_{56}\ O_4$	Negative	624.4185	623.4125	Carotenoid Ether
14	Fenothiocarb	$C_{13}\ H_{19}\ N\ O_2\ S$	Negative	253.1136	328.1217	Aromatic Ether
15	Feruloylputrescine	$C_{14}\ H_{20}\ N_2\ O_3$	Positive	264.1457	265.1532	Hydroxycinnamic Acid.
16	Feruloylputrescine	$C_{14}\ H_{20}\ N_2\ O_3$	Positive	264.1462	265.1529	Hydroxycinnamic Acid.
17	Ganoderiol I	$C_{31}\ H_{50}\ O_5$	Positive	502.3742	503.3814	Triterpenoid.
18	Gerberinol	$C_{21}\ H_{16}\ O_6$	Positive	364.0941	365.1014	Hydroxycoumarin
19	Grossamide	$C_{36}\ H_{36}\ N_2\ O_8$	Positive	624.2446	398.3389	Arylbenzofuran Flavonoids
20	L-isoleucyl-L-proline	$C_{11}\ H_{20}\ N_2\ O_3$	Positive	228.1485	251.1375	Dipeptides.
21	Lucyin A	$C_{30}\ H_{46}\ O_5$	Positive	486.3383	266.1582	Triterpenoids.
22	Miraxanthin-I	$C_{14}\ H_{18}\ N_2\ O_7\ S$	Positive	358.0865	381.0757	Tetracarboxylic Acid
23	N-(2,14-Eicosadienoyl)piperidine	$C_{25}\ H_{45}\ N\ O$	Positive	375.35	398.3394	Alkaloid
24	Nonate	$C_9\ H_{16}\ O_4$	Negative	188.1068	187.0994	Dicarboxylic Acid
25	Quercetagetin	$C_{15}\ H_{10}\ O_8$	Negative	318.0372	363.0348	Hexahydroxyflavone
26	Quinic acid	$C_7\ H_{12}\ O_6$	Negative	192.065	191.0577	Cyclohexanecarboxylic Acid
27	Resorcinol	$C_6\ H_6\ O_2$	Negative	110.0372	109.03	Isomeric Benzenediols
28	Vulgarone A	$C_{15}\ H_{22}\ O$	Positive	218.1658	219.1731	Monoterpenoids

FT-IR analysis of MEPP: The presence of bioactive chemicals predicted by HR-LCMS investigations was confirmed by FTIR analysis. The functional groups included in the *PP* methanol extract were identified using a sequence of peaks determined by FTIR analysis (Kennepohl et al., 2020), as depicted in Fig. 3. The FTIR spectrum was applied to assess the functional group of the active components based on the peak value in the infrared radiation band (Skoog *et al.,* 2017).

The FTIR analysis of PP methanolic extract reveals distinct peaks at 2945.3 cm^{-1} due to the presence of C-H stretching of polyphenolic alcohols, peak at 2360.87 cm^{-1} due to C-H bending of Alkanes, and C=C stretching at 1653 cm^{-1} due to Alkane. The presence of Carboxylic acid (O-H stretching) causes the peak at 2517.1 cm^{-1}, and the presence of aliphatic primary amine (N-H stretching) causes the peak at 3338.78 cm^{-1}. Carboxylic acid O-H bending is indicated by the peak at 1411.89 cm^{-1}. Peaks at 1112.93 and 2833.43 cm^{-1} for aliphatic ether and Alcohol, respectively, for C-O stretching and O-H stretching. The presence of Halo compounds is suggested by the peak at 632.65 cm^{-1} (C-Cl stretching) as per Table 3.

Table 3. FTIR Absorption Frequencies for Functional Groups in PP

Sr.No.	Peak	Area	Bond	Functional Group	Frequency in cm^{-1} (Intensity*)
1	632.65	10922.82	C-Cl stretching	Halo compound	690-515 (s)
2	1016.49	3438.365	C-F stretching	Fluoro compound	1400-1000 (s)
3	1112.93	1055.813	C-O stretching	Aliphatic ether	1150-1085 (s)
4	1411.89	2242.505	O-H bending	carboxylic acid	1440-1395 (m)
5	1450.47	1329.465	C-H bending	Alkane	1400 (m)
6	1653	2174.226	C=C stretching	Alkane	1662-1626 (m)
7	2360.87	194.446	C-H bending	Alkane	2250–2700 (m)
8	2517.1	269.054	O-H stretching	Carboxylic acid	3300-2500 (s)
9	2833.43	2895.286	O-H stretching	Alcohol	3200-2700 (w)
10	2945.3	5123.598	C-H stretching	Alcohol	3000-2840(m)
11	3338.78	32326.99	N-H stretching	Aliphatic primary amine	3400-3300 (m)

(s = strong; m = medium; w = weak)

As conclusion, secondary metabolites derived from natural sources are used in the effective way of producing functional food. The current study's phytochemical evaluation revealed that *PP* could be a source of therapeutic components. FTIR analysis and HR-LCMS high-resolution liquid chromatography revealed that the methanolic extract of *PP* contained functionally significant bioactive substances such as alkaloids, steroid, fatty acid, ester, phenol, hydroxycinnamic acid, Hydroxycoumarin, triterpenoids, polypeptides, glycoside, flavonoid, diterpenoid, and flavons. The findings of the samples indicate that *PP* is a great site of biologically active molecules with both structural and functional activity. Currently, research is being conducted to separate the components and incorporate the phytochemical characteristics as functional foods with nutraceutical advantages.

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Conflict of Interest: There are no conflicts of interest from the authors.

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