



Optimization of microwave-enhanced extraction parameters to recover phenolic compounds and antioxidants from *Corchorus olitorius* leaves

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Abstract

Vegetables are industrial crops endowed with both nutritional and medicinal values. The overwhelming contributions of vegetables to human living in the form of nutrients and medicine cannot be under emphasised. Thus, this study examined the recoveries of phenolic compounds and antioxidants from *Corchorus olitorius* leaves using a microwave-enhanced extraction technique. Furthermore, the phenolic compounds in the leaf extract of *C. olitorius* were comprehensively identified using liquid chromatography-mass spectrometry quadrupole of flight (LC-QToF-MS). At the optimized conditions of microwave-enhanced extraction (extraction time of 131 s, microwave power 305 W, solvent/sample ratio of 12 mL/g, and ethanol concentration of 50%), total phenolic content (TPC) of 343.098 ± 3.05 mg GAE/10 g d.b., IC₅₀ values of 68.89 ± 1.08 and 29.76 ± 1.00 µg/mL for 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6'-sulfonic acid) (ABTS) assays, respectively, were achieved. Furthermore, an aggregate of fourteen phenolic compounds that include 1-galloyl-glucose, 1,3,5-O-tricaffeoylquinic acid, procyanidin C-1, 4,4',5,6-tetrahydroxystilbene, 3,4,5-O-tricaffeoylquinic acid, 5-desgalloylstachyurin, sanguin H-4, corilagin_1, 1-O-galloylpedunculagin, laevigatin A, pedunculagin, 2,4,6-tri-O-galloyl-β-D-glucose, 1,3,6-trigalloyl-β-D-glucose, and 1,2,3,6-tetra-O-galloyl-β-D-glucose was tentatively identified in the leaf extract of *C. olitorius*. In general, this study has established *C. olitorius* leaves as sources of phenolic compounds and natural antioxidants. Thus, the intake can continue to be promoted as a way forward in solving the problem of food insecurity.

Keywords *Corchorus olitorius* · Response surface methodology · Total phenols · Antioxidant · Liquid chromatography quadrupole time-of-flight mass spectrometry · Microwave-enhanced extraction

Abbreviations

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6'-sulfonic acid)
ANOVA	Analysis of variance analysis
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FCCCD	Face-centred central composite design

LC-QToF-MS	Liquid chromatography-mass spectrometry quadrupole of flight
OFAT	One-factor-at-a-time
TPC	Total phenolic content
RSM	Response surface methodology
SDG 2	Sustainable development goals

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Introduction

Vegetables have been categorised as industrial crops with valuable means of nutrients and a source of income. Other than the valuable nutrients in vegetables, some of them possess diverse medicinal characteristics that are good for human and animal health. Although indigenous vegetables are long been employed in day-to-day diets in different communities of the world, several of these vegetables are underutilised as most have embodiments of unknown and underestimated pharmacological features. Most attention is

based on the consumption of maize, rice, and cassava. It is not disputed these crops are endowed with great nutritional values; nevertheless, vegetables can be prioritised due to their functional properties (food and medicine). Additionally, United Nation Sustainable Development Goals (SDG 2) established the importance of figuring out sustainable means of producing foods for humans, especially natural sources. This is highly needed in this time of post-Covid-19 recovery as the world is being faced with hunger, food insecurity and consumption of low-quality foods that cause micronutrient deficiencies (Guzzetti et al. 2021)

Corchorus olitorius (Jute mallow) is a leafy vegetable mostly found in Tropical regions including the Middle East, Africa and some parts of Asia (Guzzetti et al. 2021). It belongs to the family of Malvaceae (Youssef et al. 2019). This is one of the vegetables consumed mainly in different African countries including Nigeria, Tanzania, South Africa, and Ethiopia (Loumerem and Alercia 2016). Consuming this vegetable has been encouraged to curb malnutrition and food insecurity, especially in developing nations such as African countries. The consumption of *C. olitorius* leaves is much encouraged as it is believed to overcome nutritional and food insecurity among children under the ages of five, lactating mothers and pregnant women in sub-Saharan Africa (Andersen et al. 2003; Kamga et al. 2013). This vegetable is mostly consumed in the form of soup or sauce alongside solid foods from rice, yam or cassava in African cooking cuisine. It is endowed with different vitamins, enrich in fibre, and antioxidants (Guzzetti et al. 2021). A brief description of *C. olitorius*: it is an annual vegetable that can reach a height of about 2.4 m; the leaves are finely lobed or serrated margin, simple, lanceolate, and alternate; it has only a few branches and can be unbranched; seeds are used when planting; the flowers are yellowish small with five petals (2–3 cm in diameters) and can survive in any kinds of soil (loamy, sandy or clay) (Loumerem and Alercia 2016). The leaves of *C. olitorius* are enriched in beta carotene, vitamins A and C, folic acid, calcium, iron, and diverse phenolic compounds (Ndlovu et al. 2020; Youssef et al. 2019). The leaves of *C. olitorius* were reported to contain nutritional values more than stems and fruits. This is the reason why it is generally used as food. The leaves had been revealed to be made up of about seventeen active nutrients compounds such as protein, carbohydrate, fat, ash, fibre, potassium, calcium, sodium, phosphorous, iron, beta-carotene, riboflavin, thiamine, ascorbic acid, niacin, and so on. Moreover, the leaves had been reported to possess hepatoprotective, antidiabetic, antioxidant, diuretic, antipyretic, analgesic, antitumor, antimicrobial, anti-obesity, hypoglycemic, and gastroprotective properties (Abir et al. 2019; Racha et al. 2018; Saliu et al. 2019).

The presence of phenolic compounds in plant materials is continually studied due to their important link with the

occurrence of natural antioxidants that possess outstanding applications in human health (Alara et al. 2021a, b; Cheynier 2012). The antioxidants from leafy vegetables such as *C. olitorius* can act in place of synthetic antioxidants readily available in the market. These synthetic antioxidants are being reported to pose side effects on consumers (Racha et al. 2018). However, natural antioxidants can act as protection against degenerative diseases and alter the DNA, proteins and lipids (Racha et al. 2018). Besides, plants are surviving the exposure to ultraviolet radiation in the environment; this has positioned them as a perfect antioxidant source. Likewise, the consumptions of plant-sourced antioxidants are relatively safe and widely accepted; this has propelled the introduction of plant polyphenols into cosmetic and pharmaceutical products in place of synthetic antioxidants (Che Sulaiman et al. 2017; Lobo et al. 2010). The process of extraction is important when targeting to achieve significant phenolic compounds and antioxidants from plant materials. This process can either be conventional or non-conventional; the latter is currently being used due to its ability to recover a tangible quantity of phenolic compounds and antioxidants from plant materials (Alara et al. 2021a, b; Herrero et al. 2012). Out of several unconventional techniques that are being used, the microwave-enhanced method has been generally accepted due to its ability to leach a significant quantity of phenolic compounds from the considered sample within a reduced duration of time and not alter the bioactive elements in the extracts (Alara et al. 2021a, b; Alara and Abdurahman 2019).

Several parameters can alter the efficiency of recovered phenolic compounds and antioxidants from plant matrices when using microwave-enhanced technique; these are temperature, extraction time, solvent/sample ratio, solvent concentration, microwave power, and solvent selection. Out of different available solvents used in the extraction processes, ethanol and water are the most environment-friendly. It had been explained that pure ethanol cannot extract the totality of phenolic compounds especially those that are hydrophilic (water-soluble) (Thoo et al. 2010). Previous studies had suggested that the use of aqueous ethanol or binary mixture contributes to the recovery of antioxidant from plant sample (Che Sulaiman et al. 2017). Thus, mixing these two solvents (water and ethanol) in rightful proportion tends to improve the recovery of bioactive materials from plant matrices (Khoddami et al. 2013). As the selection of extraction is important in achieving significant recovery, process optimization is as well essential to achieve this goal.

There are two common techniques used for process optimization (one leading to the other); these are one-factor-at-a-time (OFAT) experimental trials and response surface methodology (RSM). Although OFAT suffers drawbacks such as no room for interaction between the factors; nonetheless, OFAT is widely employed to determine the range of

values used for the response surface methodology. RSM is utilised to settle the limitations of OFAT experimental trials which include inadequate data evaluation, being expensive and time-consuming (Che Sulaiman et al. 2017).

Although diverse studies had been carried out on *C. olitorius* leaves concerning the proximate constituents, total phenols and antioxidants (Ndlovu et al. 2020; Youssef et al. 2019). Nevertheless, there is yet a report on the optimization studies involving microwave-enhanced extraction of the total phenols and antioxidants from *C. olitorius* leaves and comprehensive identifications of the phenolic compounds. Hence, this study focuses on unveiling the optimised microwave-enhanced extraction conditions to achieve maxima phenolic compounds and antioxidants from *C. olitorius* leaves. At the optimized conditions, the comprehensive identifications of different phenolic compounds in the extracts were carried out using a fast liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS).

Materials and methods

Cultivation of *C. olitorius* seeds and sample

The seeds of *C. olitorius* were cultivated in a small garden in the Universiti Malaysia Pahang (UMP) for two months (from February and April 2019); every agricultural practice such as watering, weed control and thinning was adequately observed to achieve productive results. Sandy-loam soil was used for the cultivation. In the maturity stage, the leaves of *C. olitorius* were harvested and washed to evacuate dirt under running water. Then, the leaves were allowed to drain off the water and dried at room temperature until a constant weight was reached. The dried leaves were milled using a blender and sieved using a laboratory sieve (400/425 μm pore size). Afterwards, the ground sample was kept in a zip-lock bag and kept in a freezer at $-20\text{ }^\circ\text{C}$ before the extraction process.

Chemicals and reagents used

Ethanol, gallic acid, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, 2,2'-azino-bis(3-ethylbenzothiazoline-6'-sulfonic acid) (ABTS), potassium persulfate, and Folin–Ciocalteu reagent were procured from Sigma-Aldrich (Selangor, Malaysia).

Microwave-enhanced extraction through experimental design

The solvents used in this study were water and ethanol. An Ethos microwave oven (Milestone, Italy) was employed, and this extractor is configured using a control compartment with

power, time and temperature control. Since the microwave-enhanced extraction technique is influenced by microwave power, temperature, time, solvent concentration, and solvent/sample ratio; thus, these factors were first studied by carrying out OFAT experimental trials to evaluate the effect of an individual factor on the recovered phenolic compounds and antioxidants as well as the range for individual factor. These ranges of values were employed for the optimization studies. For the extraction, 5 g of *C. olitorius* leaf sample was adequate mixed together with an estimated proportion of ethanol–water solvent (experimental design). The mixture was filtered, and a rotary evaporator set at $50\text{ }^\circ\text{C}$ was utilised to concentrate the extract.

After the determination of preliminary ranges for the five considered factors through OFAT experimental trials, a face-centred central composite design (FCCCD) was used to optimise the recovery of phenolic compounds and antioxidants from *C. olitorius* leaves. Four factors were considered during the optimization study since temperature (kept at $40\text{ }^\circ\text{C}$) showed an insignificant effect on the dependent factors (total phenolic compounds (TPC) and antioxidant activity (DPPH and ABTS)). Thus, the independent factors were extraction time (X_1 : 60–180 s), microwave power (X_2 : 200–400 W), solvent/sample (X_3 : 8–16 mL/1 g), and ethanol concentration (X_4 : 30–50%), while the dependent factors were TPC (Y_1), DPPH (Y_2) and ABTS (Y_3). Thirty randomised runs of experiments with six centre points were obtained using FCCCD (Table S1 in supplementary materials). The regression analysis for this study was initiated using Eq. (1).

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1, i < j}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j + \sum_{i=2}^k \beta_{ii} X_i^2 \quad (1)$$

where Y indicates the dependent factors which are TPC (Y_1), DPPH (Y_2) and ABTS (Y_3), β_0 is a constant, β_i indicates the linear coefficient (actual effect), β_{ij} represents the interaction coefficients between two considered extraction factors, β_{ii} is the second-order polynomial coefficient, and k is the number of dependent factors ($k=4$). The significant values were removed, while only the insignificant values were used to construct a reduced model (Che Sulaiman et al. 2017).

Having achieved the predicted yields of the considered responses, the adequacy of the constructed models was tested. These were carried out by comparing the actual and predicted values. A pair t -test analysis was employed to verify the postulated models.

Determination of TPC in the *C. olitorius* leaf extract

The TPC in extract from *C. olitorius* leaves was estimated following the previously described procedures (Alara and Abdurahman 2019). Briefly, 200 μL of Folin–Ciocalteu reagent was added to 100 μL of leaf extract of *C. olitorius*; then,

0.2 mM Na₂CO₃ using the volume of 600 µL was added and the mixture was allowed to stay 2 h in the dark at room temperature for adequate reactions. After this, the absorbance of the reacted mixture was read at 765 nm.

Determination of antioxidant activity

DPPH analysis

The free radical scavenging potential of extracts from *C. olitorius* leaves using DPPH assay was done following the procedure previously published (Alara et al. 2018a). In brief, the DPPH solution was prepared to achieve 0.1 mM. The antioxidant activity of the extract from *C. olitorius* leaves was presented as IC₅₀ (this is the concentration of the *C. olitorius* leaf extract needed to inhibit 50% formation of DPPH radicals). These were done by preparing four different concentrations for each run of extract (0.1, 0.2, 0.3, and 0.5 mg/mL). From each concentration, 200 µL of the extract and 0.1 mM DPPH solution (2 mL volume) were thoroughly mixed. The absorbance was read at 517 nm after the mixture was left to adequate react for 30 min. IC₅₀ was determined from the linear graph between the percentage of inhibition and extract concentration ($y = 0.1478x + 19.781$, $R^2 = 0.9986$).

ABTS analysis

For the ABTS analysis, the free radical scavenging potential of extracts from *C. olitorius* leaves using DPPH assay was done following the procedure previously published (Alara et al. 2018a). In brief, an equal ratio of 2.45 mM potassium persulfate and 7 mM ABTS⁺ solution was dissolved in 120 mL of methanol to achieve the stock solution of ABTS. This stock solution was allowed to react for 12 h before its usage. Then, 2 mL of stock solution was further diluted in 120 mL of methanol to obtain a fresh solution with an absorbance of 1.10 ± 0.02 at 734 nm. The antioxidant activity of the extract from *C. olitorius* leaves using ABTS was presented as IC₅₀. These were done by preparing four different concentrations for each run of extract (0.1, 0.2, 0.3, and 0.5 mg/mL). From each concentration, 2.85 mL of a fresh solution of ABTS and 0.15 mL of extract were thoroughly mixed. At 734 nm, the absorbance was recorded after the mixture had been left to react for 120 min. IC₅₀ was determined from the linear graph between the percentage of inhibition and extract concentration ($y = 0.1008x + 46.858$, $R^2 = 0.9977$).

LC-QToF-MS analysis of phenolic compounds

Every phenolic compound in the leaf extract of *C. olitorius* at the optimized microwave-enhanced extraction

conditions was identified using LC-QToF-MS (Waters Vion IMS, USA). A C₁₈ column with the dimension of 100 mm × 2.10 mm × 1.80 µm was used. There are two mobile phases used in this analysis; they are water + 0.1% formic acid (A) and 100% acetonitrile (B). The operating conditions were a seal wash period of 5 min, a pre-injector volume of 0 µL, low pressure of 0 psi, high-pressure limit of 18,000 psi, gradient elution was 90% A and 10% B (1.25 min), 45% A and 55% B (4.17 min), 10% A and 90% B (6.25 min), 90% A and 10% B (8.34 min), with the injection volume of 20 µL, the flow rate of 0.50 mL/min, spray voltage of 4 keV, mass range of 100–1000 m/z, sample temperature of 15 °C, column temperature of 40 °C, gas flow of 0.50 mL/min, desolvation gas flow rate of 800 L/h, desolvation temperature of 550 °C, low collision energy of 4 eV, high collision energy ramp between 10 and 45 eV, source temperature of 120 °C. The identifications of phenolic compounds in the leaf extract of *C. olitorius* were performed by utilising SYNAPT mass spectrometer (Waters) coupled with an electrospray ionization operated in negative ion mode. Waters® UNIFY Software 1.0.0 installed in equipment was used to identify the phenolic compounds.

Data analysis

Each experimental trial was repeated thrice, and the data were recorded as mean values ± standard deviation. The Design Expert® software (Version 7, Stat. Ease Inc., Minneapolis, USA) was employed to carry out the optimization study. The model significance was based on a *p* value less than 0.05 and vice versa for the insignificant model. Three-dimensional plots were used to understand the interactions between two factors. A Chi-squared test was used to verify the validity of the predicted models.

Results and discussions

One-factor-at-a-time experimental trials for the extraction of TPC and antioxidants from *C. olitorius* leaves

From the beginning of this study, the preliminary experimental trials were to study the effects of the factors influencing the recoveries of phenolic compounds and antioxidants from *C. olitorius* leaves using a microwave-enhanced extraction technique. These factors were extraction time, microwave power, solvent/sample ration, ethanol concentration, and temperature. Likewise, the experimental ranges for the subsequent optimization study were determined using OFAT. OFAT experimental trials involve varying a factor, while others are kept constant.

Contributing impact of extraction time

Extraction time is an essential factor when considering solid–liquid extraction; this is due to its impact on mass transfer and solubility. Figure 1Ai and Aii showed the impact of extraction time on the recoveries of phenolics and antioxidants from *C. olitorius*. This was done by keeping solvent/sample ratio, microwave power, ethanol concentration, and temperature at 8 mL/g, 200 W, 20%, and 20 °C, respectively. It can be observed that TPC recovery increased with extraction time to achieve a higher yield at 120 s before the decline in the yield set in. This might be because the extended time of extraction can be dangerous to phenolic compounds in the plant matrix; leading to degradation when exposed to microwave radiation (Alara et al. 2021b; Azwanida 2015). On contrary, the recoveries of antioxidant through DPPH and ABTS declined with increasing time; the minima yield of antioxidants was achieved at 120 s prior to the further increase in the yield with increasing extraction time. This decrease occurs because the antioxidant activity is inversely proportional to IC₅₀ values (linear regression between the percentage of inhibition and extract concentration) (Mahmud et al. 2019). Thus, a lesser IC₅₀ value indicates higher antioxidant potential. The maxima scavenging potentials of *C. olitorius* leaves for both DPPH and ABTS were reached at 120 s. For the optimization study, 60–180 s was the range of values. However, 120 s was considered as the fixed extraction time when studying the impacts of other factors.

Contributing impact of solvent/sample ratio

During the solid–liquid extraction, the solvent/sample ratio can impact the recoveries of phenolic compounds and antioxidants. This is because this factor has an impact on the concentration gradient between the solutes in the plant sample and the solvent (Tchabo et al. 2018). In this study, the weight of the *C. olitorius* leaves was kept constant, while the volume of the solvent was varied. The effect of solvent/sample ratio was studied by keeping extraction time, microwave power, ethanol concentration, and temperature at 120 s, 200 W, 20%, and 20 °C, respectively. As seen in Fig. 1Bi, the recovery of TPC tends to increase as the solvent/sample ratio improves until the maximum recovery of TPC was obtained when the solvent/sample ratio reached 12 mL/g. Beyond the solvent/sample ratio of 12 mL/g, the occurrence of phenolic compounds in the leaf extract of *C. olitorius* declined. For the maxima, antioxidant potentials through DPPH and ABTS (Fig. 1Bii); the lowest IC₅₀ values (strong antioxidant) were reached at a solvent/sample ratio of 12 mL/g. Beyond 12 mL/g, the antioxidant potentials declined with increasing solvent volume. This is industrially valuable because reducing solvent usage that achieves higher recovery favours the production cost (Alara et al. 2018a; Bouras et al. 2015; Tchabo

et al. 2018). Moreover, the higher solvent volume tends to increase the concentration gradient; this can then lead to an increased diffusion rate of solute into the solvent. Therefore, 8–16 mL/g was chosen as a range of values for the optimization. However, 12 mL/g was considered as the fixed solvent/sample ratio when studying the impacts of other factors.

Contributing impact of microwave power

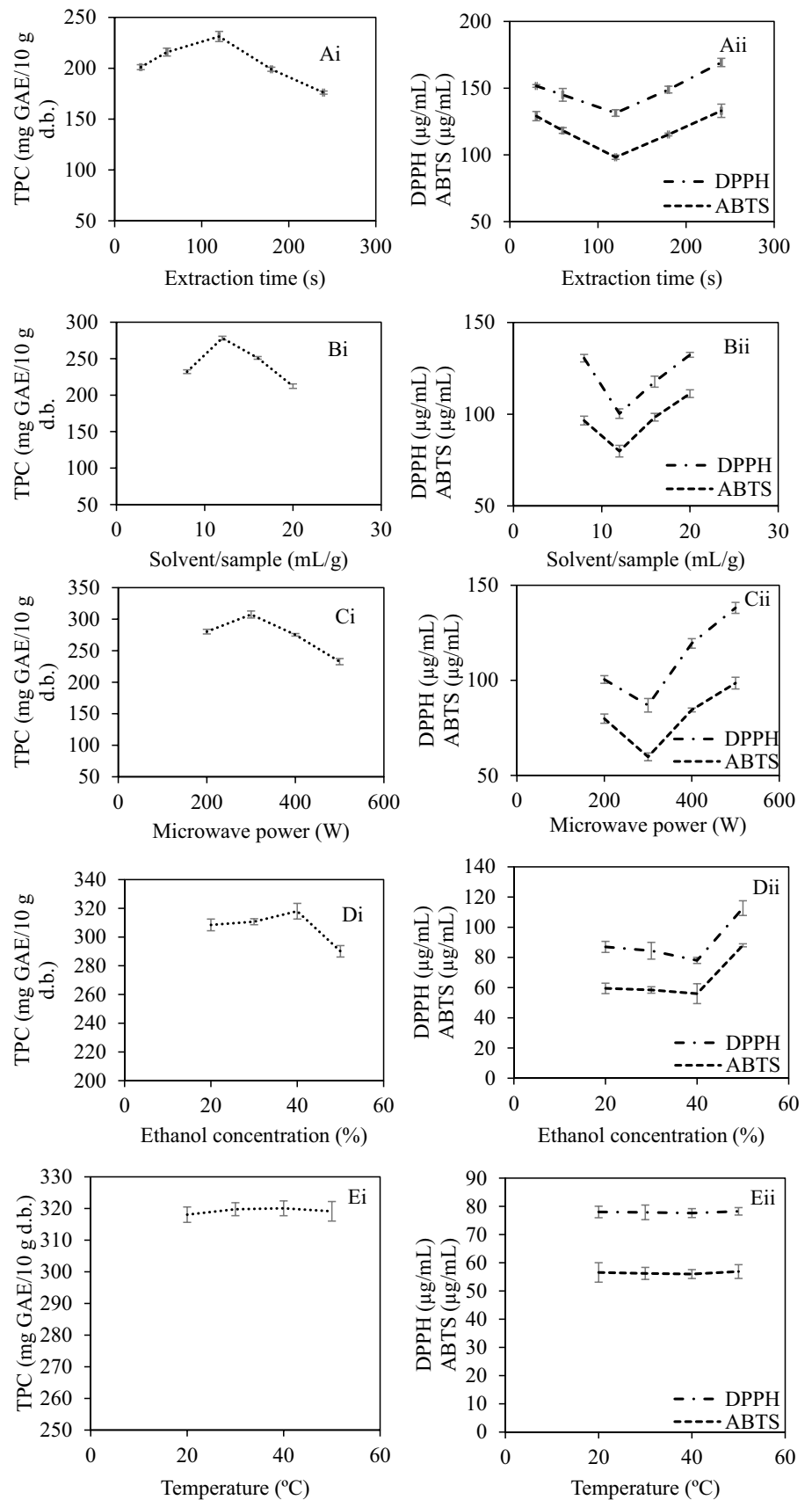
In microwave-enhanced extraction, the plant sample absorbs microwave energy, this energy changes to heat and evaporation kick-starts from the left-over moisture content in the plant matrix. The evaporated water then causes the pressure build-up within the cell wall, leading to cell rupture in the plant sample. Thus, the bioactive compounds within the plant matrix will leach out into the surrounding solvent after cell rupture (Mandal et al. 2007).

In this, the impact of microwave power on the recoveries of phenolic compounds and antioxidants from *C. olitorius* leaves was investigated by fixing the following factors: extraction time (120 s), solvent/sample ratio (12 mL/g), ethanol concentration (20%), and temperature (20 °C) (Fig. 1Ci and Cii). There was a slight increase in the TPC recovery as the power increased. At microwave power of 300 W, maximum TPC was achieved, after which the recovery of phenolic compounds tends to decrease against increasing microwave power. In contrast, the antioxidants (IC₅₀ values) for both DPPH and ABTS declined with increasing microwave power. This is justifiable because a lower IC₅₀ value indicates higher antioxidant potential. The minima IC₅₀ values for DPPH and ABTS were obtained at 300 W of microwave power. Beyond 300 W, the phenolic compounds and antioxidants declined with increasing power. Increasing the microwave power can propel increasing temperature. At higher temperatures, the viscosity and surface tension of extracting solvent will be reduced as the diffusion coefficient and solubility increase. Nonetheless, overexposure of the bioactive compounds already leached out into the solvent to higher microwave power propels the degradation of these phenolic compounds and antioxidants (thermal-sensitive phytochemicals) (Quoc 2021). Thus, 200–400 W was selected as a range of values for the optimization. Nevertheless, 300 W was considered as the fixed microwave power when studying the remaining factors.

Contributing impact of ethanol concentration

Organic solvents are mostly used in the extraction of phenolic compounds and antioxidants from plant materials. These solvents are mostly mixed with water because it enhances the water-soluble phytochemicals in the plant sample. Moreover, the mixture of organic solvents with water tends to create a moderate polar medium. Out of several

Fig. 1 Multiple parameters that influence the recoveries from phenolics and antioxidants from *C. olitorius* leaves



organic solvents used in extraction, ethanol has been considered safe because it is environment-friendly (Alara et al. 2018a).

The influence of ethanol concentration was investigated by fixing extraction time, microwave power, solvent/sample ratio, and temperature at 120 s, 300 W, 12 mL/g, and 20 °C, respectively. As provided in Fig. 1Di and Dii, the recovery of TPC tends to increase and IC₅₀ values (showing higher antioxidants) declined as ethanol concentration in water increased until the maximum recoveries of TPC and antioxidants (both DPPH and ABTS) were achieved ethanol concentration of 40%. Beyond this concentration, the phenolic compounds and antioxidants in the leaf extract of *C. olitorius* declined. Hence, the ethanol concentration ranged between 30 and 50% for the optimization. However, 40% was considered the fixed concentration for the next factor.

Contributing impact of temperature

In solid–liquid extraction, the higher temperature tends to upsurge the release of phenolic compounds and antioxidants from the plant matrix. This is due to its influence on mass transfer (diffusion coefficient), equilibrium (solubility) and stability of phytochemicals (Tchabo et al. 2018). Although, heating can soften the plant tissue and weaken the interactions of phenolic compounds; hence, it promotes the migration of bioactive compounds into the extracting solvent. Nonetheless, a higher temperature can cause degradation and oxidation of phenolic compounds and antioxidants in the plant sample.

At fixed extraction time (120 s), microwave power (300 W), solvent/sample ratio (12 mL/g), and an ethanol concentration of 40%, the impact of the temperature was as well studied. In Fig. 1Ei and Eii, it can be observed that temperature did not influence the recoveries of phenolic compounds and antioxidants from *C. olitorius* leaves. This might be because the increasing temperature beyond a certain limit can profuse solvent loss through augment and vaporization; leading to the destruction of the phenolic compounds and antioxidants (Alara et al. 2018a; Tchabo et al. 2018).

Optimization study and modelling of microwave-enhanced extraction of *C. olitorius* leaves

Model fittings

The results from OFAT experimental trials have established the impacts of extraction time, solvent/sample ratio, microwave power, and ethanol concentration. These four factors were optimized through microwave-enhanced extraction to recover higher yields of phenolic compounds and antioxidants from *C. olitorius* leaves. In this study, FCCCD was

employed to optimize this process. This was used because it is the simplest form of RSM. A total of 30 experimental trials were generated (Table 1). The obtained outcomes from the randomized experimental trials are presented in Table 1. These results were further analysed using analysis of variance to investigate the contributing impacts of individual factors considered and their interactions. The effectiveness of the RSM model is best explained through the satisfaction of model performance by measuring the determination coefficients (R^2), F -values, lack of fit, coefficient of variation, adjusted R^2 , adequate precision, and p values (the value less than 0.05 indicates significant and vice versa).

Table 2 presents the results obtained for the analysis of variance in recovering phenolic compounds and antioxidants (DPPH and ABTS assays). It can be observed that the three models of the responses (TPC, DPPH and ABTS assays) are significant. Out of the four considered factors for the optimization study, microwave power and ethanol concentration are the most significant with a p value < 0.0001, showing model significance at a 99.9% confidence level. Extraction time is also significant, whereas solvent/sample ratio was insignificant for the three responses. Moreover, the lack of fit for the models was far greater than 0.05; this shows that the models are significant. The R^2 values of the TPC, DPPH and ABTS assays ranged between 0.98 and 0.99; these are indications that the predictive models can explain at least 98% of the result variations. The adjusted and predicted R^2 values for the three predictive models were closer to 1, indicating significant correlations between the actual and predicted results (Alara et al. 2021a). The lower value of the coefficient of variation below 10% shows that the experimental results were reproducible and reliable. The obtained results indicate for CV values for TPC, and antioxidant assays (DPPH and ABTS) were 0.33, 2.04 and 3.44, respectively. It should be noted that the contributions of the quadratic models are notable. Thus, the predictive models for the phenolic compounds and antioxidant assays are well suitable for the prediction.

Effects of microwave-enhanced extraction factors on TPC and antioxidant assays (DPPH and ABTS) in *C. olitorius* leaves

The influences of microwave-enhanced extraction factors on the phenolic compounds and antioxidants from *C. olitorius* leaves. The phenolic compounds in the extract of *C. olitorius* leaves varied from 325.52 ± 2.99 to 346.89 ± 2.17 mg GAE/10 g d.b. (Table 2). The least TPC was obtained at extraction time of 180 s, microwave power of 400 W, solvent/sample ratio of 16 mL/g, and ethanol concentration of 50%, whereas the highest TPC was obtained at extraction time of 120 s, microwave power of 200 W, solvent/sample ratio of 12 mL/g, and ethanol concentration of 40%. The antioxidant assays (DPPH and ABTS) from the leaf extract

Table 1 Design of experiments for the optimization of microwave-enhanced extraction of TPC and antioxidants from *C. olitorius* leaves

Std	Extraction time, X_1 (s)	Microwave power, X_2 (W)	Solvent/sample, X_3 (mL/g)	Ethanol concentration, X_4 (%)	TPC, Y_1 (mg GAE/10 g d.b.)	DPPH, Y_2 ($\mu\text{g/mL}$)	ABTS, Y_3 ($\mu\text{g/mL}$)
1	60	200	8	30	325.69 \pm 4.07	113.79 \pm 2.00	74.13 \pm 1.08
2	180	200	8	30	327.00 \pm 2.78	109.47 \pm 2.12	70.24 \pm 1.05
3	60	400	8	30	326.42 \pm 4.29	125.98 \pm 1.16	86.68 \pm 2.43
4	180	400	8	30	327.72 \pm 3.00	108.50 \pm 2.56	69.22 \pm 1.54
5	60	200	16	30	327.50 \pm 2.55	120.00 \pm 1.06	80.37 \pm 1.58
6	180	200	16	30	326.33 \pm 1.09	119.60 \pm 2.14	80.47 \pm 1.99
7	60	400	16	30	329.92 \pm 4.76	117.00 \pm 1.02	77.79 \pm 2.19
8	180	400	16	30	325.14 \pm 4.11	103.72 \pm 2.55	64.50 \pm 1.56
9	60	200	8	50	342.17 \pm 2.33	120.02 \pm 2.28	80.83 \pm 1.05
10	180	200	8	50	341.89 \pm 2.36	99.92 \pm 3.05	60.60 \pm 2.11
11	60	400	8	50	336.78 \pm 2.56	94.48 \pm 1.16	55.25 \pm 1.55
12	180	400	8	50	330.63 \pm 2.00	70.67 \pm 1.65	31.36 \pm 1.00
13	60	200	16	50	344.55 \pm 2.11	120.15 \pm 2.04	81.00 \pm 1.05
14	180	200	16	50	340.18 \pm 2.23	107.99 \pm 1.00	68.66 \pm 1.00
15	60	400	16	50	332.82 \pm 2.05	90.45 \pm 2.09	51.22 \pm 1.02
16	180	400	16	50	325.52 \pm 2.99	70.29 \pm 1.77	31.08 \pm 1.03
17	60	300	12	40	343.47 \pm 4.22	101.04 \pm 1.22	61.81 \pm 1.06
18	180	300	12	40	342.78 \pm 3.08	85.28 \pm 2.06	46.00 \pm 1.08
19	120	200	12	40	346.89 \pm 2.17	102.02 \pm 2.09	62.82 \pm 1.00
20	120	400	12	40	342.51 \pm 2.65	85.20 \pm 1.18	45.96 \pm 0.99
21	120	300	8	40	342.70 \pm 2.65	102.39 \pm 2.35	63.10 \pm 1.69
22	120	300	16	40	339.54 \pm 1.98	104.14 \pm 1.23	64.93 \pm 2.01
23	120	300	12	30	333.32 \pm 4.03	90.82 \pm 2.55	51.59 \pm 1.03
24	120	300	12	50	344.55 \pm 1.00	68.84 \pm 1.34	29.58 \pm 1.03
25	120	300	12	40	344.00 \pm 3.11	86.40 \pm 2.07	47.30 \pm 1.26
26	120	300	12	40	345.74 \pm 2.16	90.08 \pm 2.02	50.84 \pm 1.00
27	120	300	12	40	345.91 \pm 2.78	90.26 \pm 1.02	51.01 \pm 1.00
28	120	300	12	40	345.68 \pm 3.27	90.01 \pm 1.15	50.78 \pm 1.22
29	120	300	12	40	345.77 \pm 1.97	90.09 \pm 1.04	50.84 \pm 2.14
30	120	300	12	40	345.92 \pm 2.00	90.23 \pm 2.22	51.00 \pm 1.14

of *C. olitorius* ranged from 68.84 ± 1.34 to 125.98 ± 1.16 $\mu\text{g/mL}$ and 29.58 ± 1.03 to 86.68 ± 2.43 $\mu\text{g/mL}$, respectively. The least (extraction time of 60 s, microwave power of 400 W, solvent/sample ratio of 8 mL/g, and ethanol concentration of 30%) and highest (extraction time of 120 s, microwave power of 300 W, solvent/sample ratio of 12 mL/g, and ethanol concentration of 50%) yields of antioxidants from *C. olitorius* leaves were obtained. Analysis of variance analysis (ANOVA) results indicate that extraction time, microwave power and ethanol concentration significantly influenced the recoveries of phenolic compounds from *C. olitorius* leaves; however, the solvent/sample ratio was insignificant. The quadratic effects of all the factors except microwave power are significant (p value < 0.05). Besides, the interactions between all the considered factors significantly impact the microwave-enhanced extraction of phenolic compounds and antioxidants from *C. olitorius* leaves except for the

interactions between solvent/sample ratio and ethanol concentration (X_3X_4) on the recoveries of antioxidants from *C. olitorius* leaves which were insignificant (p value > 0.05). The quadratic model for the coded values of TPC, DPPH and ABTS assays is estimated from Eqs. (2–4).

$$Y_1 = 345.43 - 1.23X_1 - 2.49X_2 - 0.53X_3 + 5X_4 - 0.78X_1X_2 - 0.86X_1X_3 - 0.92X_1X_4 - 0.62X_2X_3 - 2.86X_2X_4 - 0.65X_3X_4 - 2.23X_1^2 - 0.66X_2^2 - 4.24X_3^2 - 6.42X_4^2 \quad (2)$$

$$Y_2 = 88.89 - 7.08X_1 - 8.15X_2 + 0.45X_3 - 9.23X_4 - 2.36X_1X_2 + 1.23X_1X_3 - 2.55X_1X_4 - 2.67X_2X_3 - 7.16X_2X_4 + 0.076X_3X_4 + 4.89X_1^2 + 5.34X_2^2 + 14.99X_3^2 - 8.44X_4^2 \quad (3)$$

Table 2 Analysis of variance for the optimization of microwave-enhanced extraction of TPC and antioxidants from *C. olitorius* leaves

TPC							DPPH							ABTS						
Source	Sum of squares	DF	Mean square	F-value	p value Prob > F	Source	Sum of squares	DF	Mean square	F-value	p value Prob > F	Source	Sum of squares	DF	Mean square	F-value	p value Prob > F			
Model	1856.45	14	132.60	105.49	<0.0001	Model	6946.43	14	496.17	121.69	<0.0001	Model	6922.90	14	494.49	117.32	<0.0001			
X ₁ : Extraction time	27.21	1	27.21	21.64	0.0003	X ₁ : Extraction time	902.70	1	902.70	221.39	<0.0001	X ₁ : Extraction time	895.35	1	895.35	212.43	<0.0001			
X ₂ : Microwave power	111.20	1	111.20	88.47	<0.0001	X ₂ : Microwave power	1195.12	1	1195.12	293.10	<0.0001	X ₂ : Microwave power	1185.20	1	1185.20	281.19	<0.0001			
X ₃ : Solvent/sample ratio	5.01	1	5.01	3.99	0.0643	X ₃ : Solvent/sample ratio	3.66	1	3.66	0.90	0.3582	X ₃ : Solvent/sample ratio	4.12	1	4.12	0.98	0.3386			
X ₄ : Ethanol concentration	450.50	1	450.50	358.38	<0.0001	X ₄ : Ethanol concentration	1532.18	1	1532.18	375.77	<0.0001	X ₄ : Ethanol concentration	1520.03	1	1520.03	360.63	<0.0001			
X ₁ X ₂	9.64	1	9.64	7.67	0.0143	X ₁ X ₂	89.07	1	89.07	21.84	0.0003	X ₁ X ₂	92.26	1	92.26	21.89	0.0003			
X ₁ X ₃	11.90	1	11.90	9.47	0.0077	X ₁ X ₃	24.28	1	24.28	5.95	0.0276	X ₁ X ₃	24.50	1	24.50	5.81	0.0292			
X ₁ X ₄	13.62	1	13.62	10.83	0.0049	X ₁ X ₄	103.79	1	103.79	25.45	0.0001	X ₁ X ₄	110.57	1	110.57	26.23	0.0001			
X ₂ X ₃	6.20	1	6.20	4.93	0.0422	X ₂ X ₃	114.01	1	114.01	27.96	<0.0001	X ₂ X ₃	113.53	1	113.53	26.94	0.0001			
X ₂ X ₄	130.64	1	130.64	103.93	<0.0001	X ₂ X ₄	819.82	1	819.82	201.06	<0.0001	X ₂ X ₄	828.86	1	828.86	196.65	<0.0001			
X ₃ X ₄	6.84	1	6.84	5.44	0.0340	X ₃ X ₄	0.092	1	0.092	0.022	0.8829	X ₃ X ₄	0.070	1	0.070	0.017	0.8990			
X ₁ ²	12.91	1	12.91	10.27	0.0059	X ₁ ²	61.94	1	61.94	15.19	0.0014	X ₁ ²	61.19	1	61.19	14.52	0.0017			
X ₂ ²	1.12	1	1.12	0.89	0.3605	X ₂ ²	73.87	1	73.87	18.12	0.0007	X ₂ ²	74.01	1	74.01	17.56	0.0008			
X ₃ ²	46.51	1	46.51	37.00	<0.0001	X ₃ ²	582.53	1	582.53	142.87	<0.0001	X ₃ ²	580.61	1	580.61	137.75	<0.0001			
X ₄ ²	106.86	1	106.86	85.01	<0.0001	X ₄ ²	184.58	1	184.58	45.27	<0.0001	X ₄ ²	185.44	1	185.44	44.00	<0.0001			
Residual	18.86	15	1.26			Residual	61.16	15	4.08			Residual	63.22	15	4.21					
Lack of fit	16.10	10	1.61	2.92	0.1243	Lack of fit	49.50	10	4.95	2.12	0.2103	Lack of fit	52.42	10	5.24	2.42	0.1701			
Pure error	2.76	5	0.55			Pure error	11.66	5	2.33			Pure error	10.81	5	2.16					
Correlation total	1875.30	29				Correlation total	7007.59	29				Correlation total	6986.12	29						

Table 2 (continued)

TPH						DPPH						ABTS					
Source	Sum of squares	DF	Mean square	F-value	p value Prob > F	Source	Sum of squares	DF	Mean square	F-value	p value Prob > F	Source	Sum of squares	DF	Mean square	F-value	p value Prob > F
Coefficient of variation (%)	0.33					Coefficient of variation (%)	2.04					Coefficient of variation (%)	3.44				
PRESS	118.47					PRESS	368.81					PRESS	389.33				
R-square	0.9899					R-square	0.9913					R-square	0.9910				
Adjusted R-square	0.9806					Adjusted R-square	0.9831					Adjusted R-square	0.9825				
Predicted R-square	0.9368					Predicted R-square	0.9474					Predicted R-square	0.9443				
Adequate precision	28.799					Adequate precision	39.281					Adequate precision	38.612				

DF degree of freedom

$$\begin{aligned}
 Y_3 = & 49.67 - 7.05X_1 - 8.11X_2 + 0.48X_3 - 9.19X_4 - 2.40X_1X_2 \\
 & + 1.24X_1X_3 - 2.63X_1X_4 - 2.66X_2X_3 - 7.20X_2X_4 \\
 & + 0.066X_3X_4 + 4.86X_1^2 + 5.34X_2^2 + 14.97X_3^2 - 8.46X_4^2
 \end{aligned}
 \tag{4}$$

where X_1 represents extraction time (s), X_2 indicates microwave power (W), X_3 represents solvent/sample ratio (mL/g), X_4 is the ethanol concentration, Y_1 , Y_2 and Y_3 are the responses TPC, DPPH and ABTS assays, accordingly.

The three-dimensional plot is the representation of regression equations to show the interaction between independent and dependent factors. This plot is presented in different shapes; this illustrates whether the factor or its mutual interaction is significant or not (Halee et al. 2020). The contributing impacts of individual factors and their mutual interactions on the phenolic compounds and antioxidants are presented using three-dimensional graphical representations in Figs. 2a–f, 3a–f and 4a–f. It should be noted that antioxidant increases with reduced IC_{50} values (Mahmud et al. 2019).

The contributing effect of interactions between microwave power and extraction time on TPC at a fixed solvent/sample ratio of 12 mL/g and ethanol concentration of 40% is shown in Fig. 2a. A gradual increase in extraction time and a slight decline in microwave power slowly increase the yield of TPC. Nonetheless, beyond 131.37 s and 305.08 W, TPC started to decline. For Fig. 2b, increasing both solvent/sample and extraction time at a fixed microwave power of 300 W and ethanol concentration of 40% gradually increased the recovery of TPC from *C. olistorius* leaves. After the certain limits of microwave power of 305.08 W and extraction time of 131.37 s, the phenolic compounds declined. In Fig. 2c, TPC from *C. olistorius* leaves increases with increasing ethanol concentration and reduces the extraction time. After these factors reached 45.07% and 69.85 s, respectively, TPC gradually decreases. The interaction between solvent/sample ratio and microwave power is presented in Fig. 2d; increasing solvent/sample ratio, and reducing microwave power increased the recovery of phenolic compounds. At solvent/sample ratio and microwave power of 11.48 mL/g and 257.62 W, respectively, the recovery of TPC decreased. For the interaction between ethanol concentration and extraction time (Fig. 2c), increasing ethanol concentration and reducing microwave power favour increasing phenolic compounds (Fig. 2e). At the ethanol concentration and microwave power of 45.07% and 257.62 W, optimal yields of TPC and antioxidants were achieved; beyond these values, the recoveries declined. Finally, the effects of interactions between ethanol concentration and solvent/sample ratio on the recoveries of TPC from *C. olistorius* leaves (Fig. 2f). Reducing the solvent/sample ratio and increasing the ethanol concentration favour the recovery of optimal TPC from the considered sample. Hence, the optimal predicted TPC yield of 343.327 mg

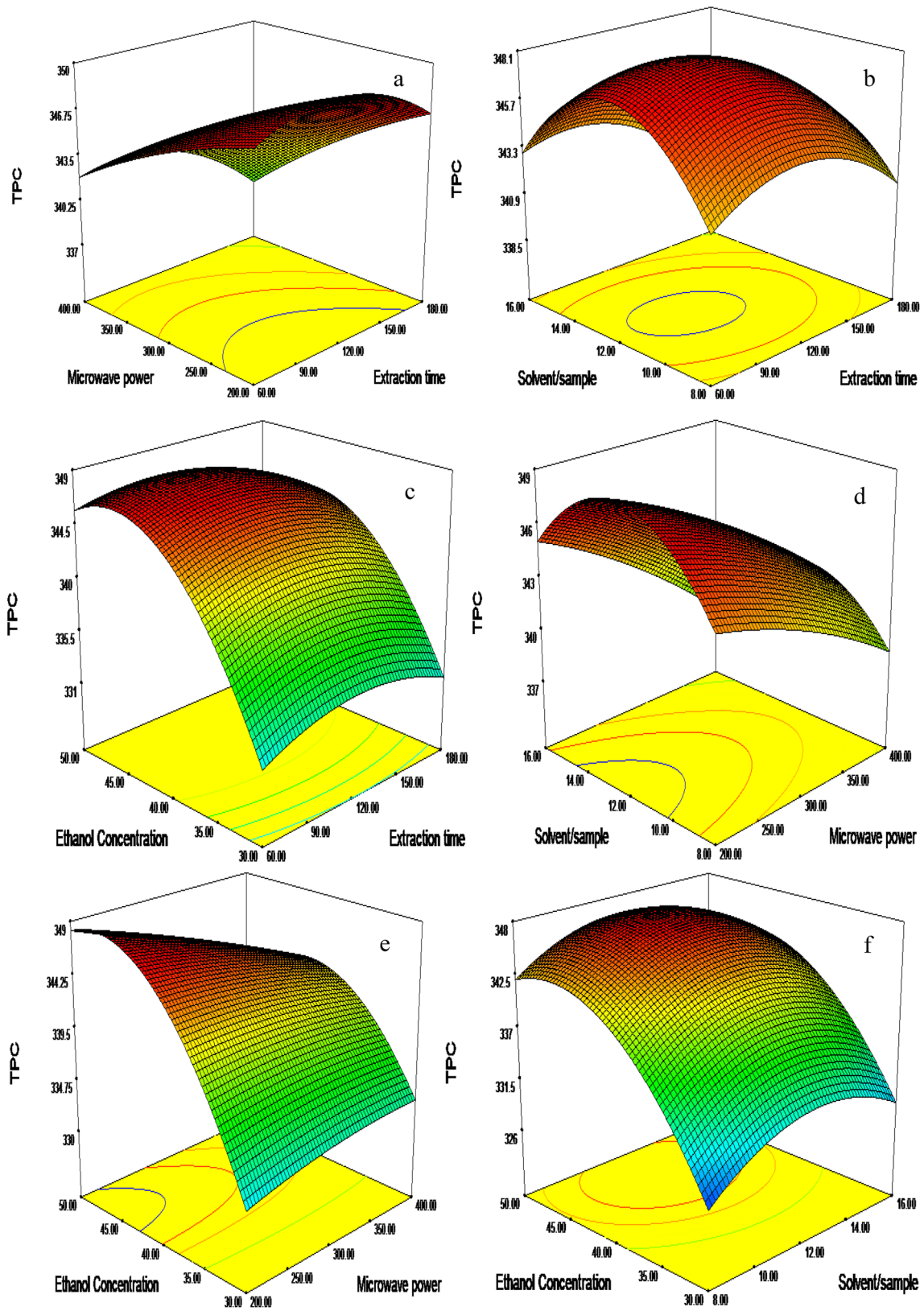


Fig. 2 Contributing impacts of individual factor and their mutual interactions on the phenolic compounds from *C. olitorius* leaves

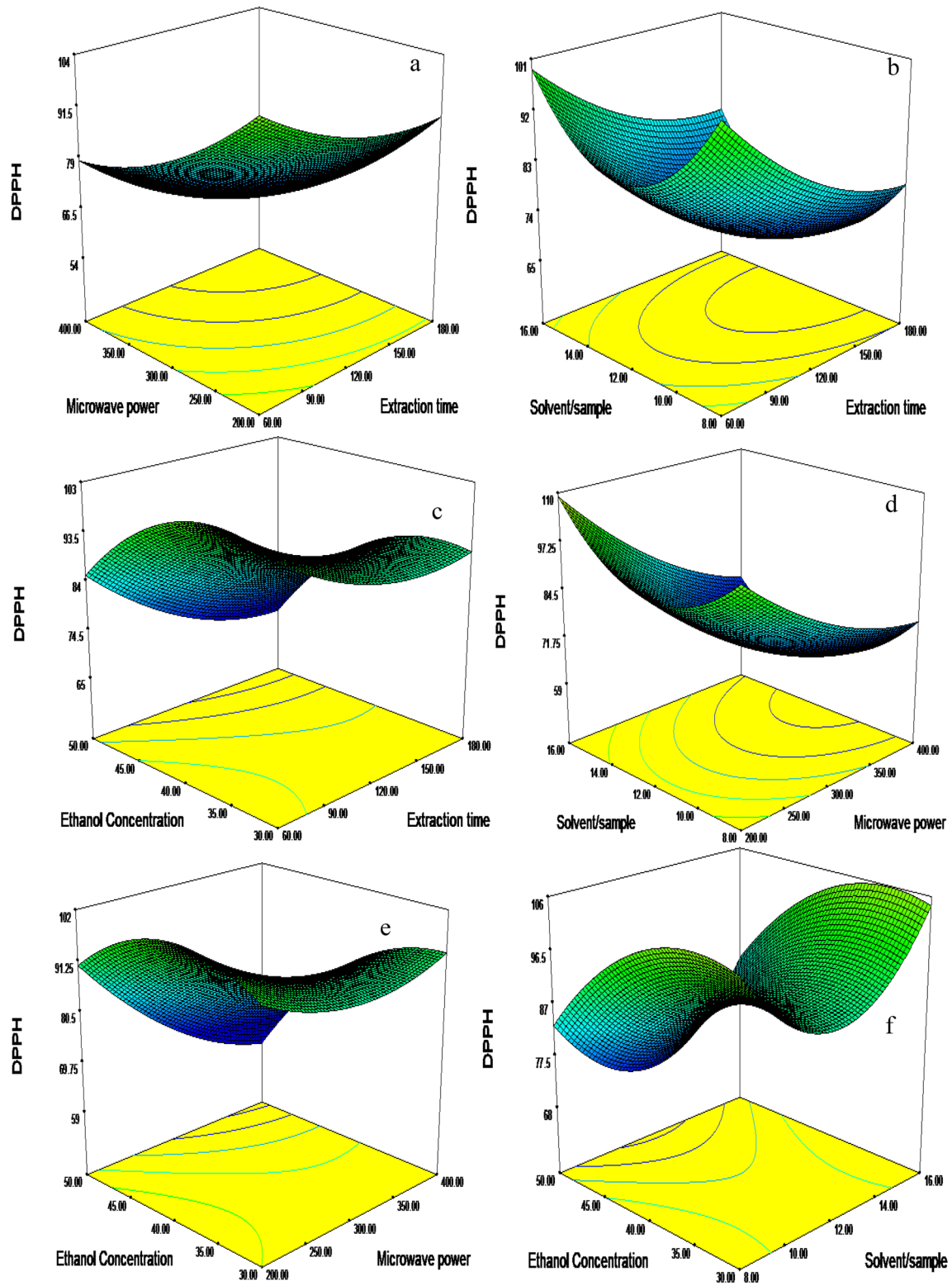


Fig. 3 Contributing impacts of individual factor and their mutual interactions on the DPPH assay in recovering antioxidants from *C. olitorius* leaves

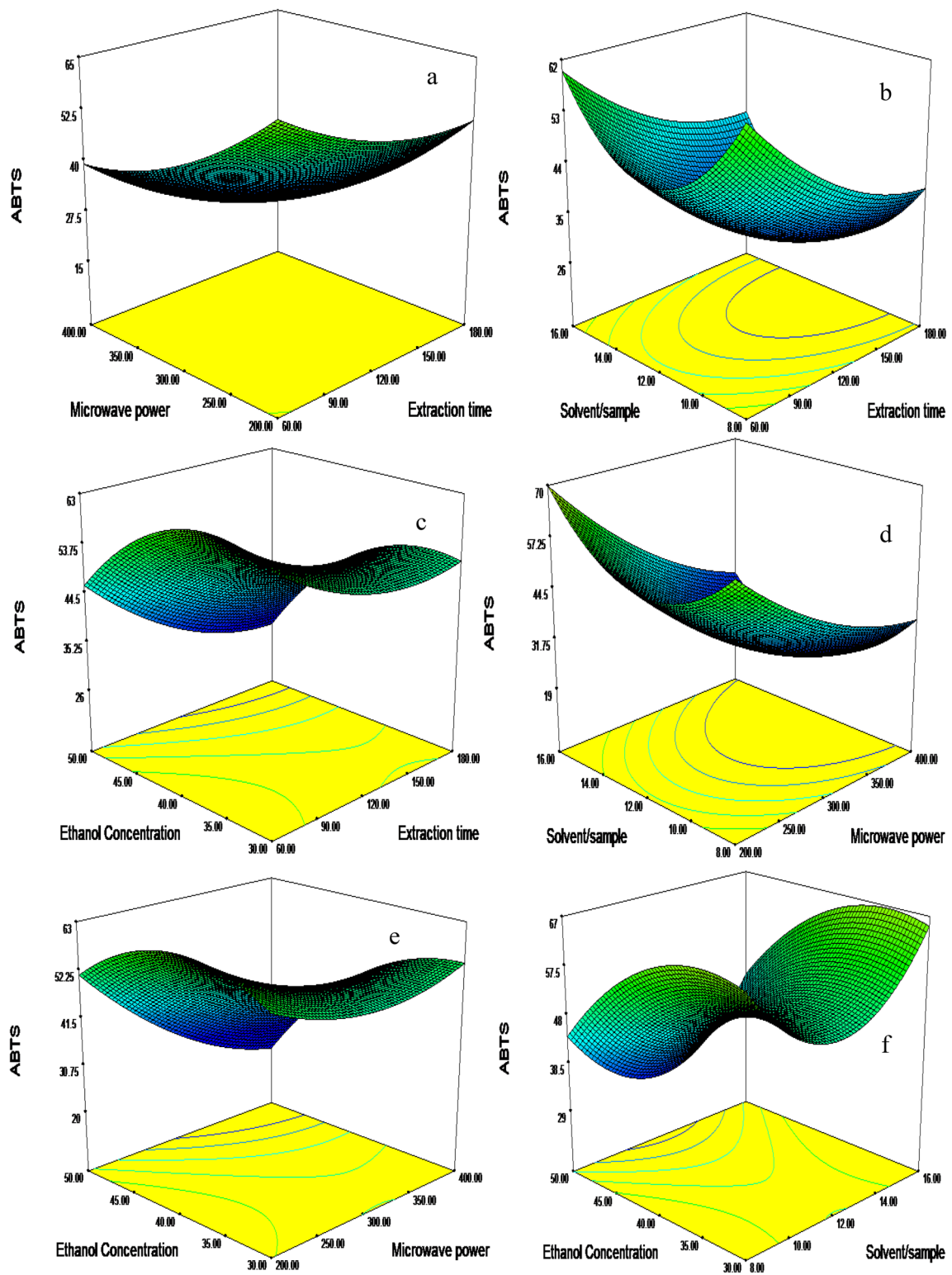


Fig. 4 Contributing impacts of individual factor and their mutual interactions on the ABTS assay in recovering antioxidants from *C. olitorius* leaves

GAE/10 g d.b. at the microwave-enhanced extraction conditions including extraction time, microwave power, solvent/sample ratio, and ethanol concentration at 131.37 s, 305.08 W, 11.67 mL/g, and 50%, respectively.

Moreover, the influence of interactions between microwave power and extraction time on antioxidants (DPPH and ABTS assays) in the leaf extract of *C. olitorius* at a fixed solvent/sample ratio of 12 mL/g and ethanol concentration of 40% is presented in Figs. 3a and 4a. Increasing the extraction time and a slight decrease in microwave power gradually increases the antioxidant yields. The antioxidants in leaf extract declined as the microwave power and extraction time reached 131.37 s and 305.08 W, respectively. Besides, the interaction between solvent/sample ratio and extraction time is presented in Figs. 3b and 4b showed that increasing solvent/sample ratio and declining extraction time favoured antioxidant recoveries from *C. olitorius* leaf at a fixed microwave power of 300 W and ethanol concentration of 40%. Beyond microwave power of 305.08 W and extraction time of 131.37 s, the antioxidants declined. For Figs. 3c and 4c, the antioxidants increase with increasing ethanol concentration and extraction time; beyond 45.07% and 69.85 s, respectively, gradual declination of antioxidants was observed. Increasing solvent/sample ratio and reducing microwave power increased the recoveries of phenolic compounds (Figs. 2d and 3d). On reaching solvent/sample ratio and microwave power of 11.48 mL/g and 257.62 W, respectively, the recoveries of antioxidants declined. Similarly, in Figs. 3c and 4c, increasing ethanol concentration and reducing microwave power increase the recoveries of antioxidants from leaf extract of *C. olitorius* (Figs. 3e and 4e). Beyond the ethanol concentration and microwave power of 45.07% and 257.62 W, respectively, the recoveries of antioxidants declined. In conclusion, the interactions between ethanol concentration and solvent/sample ratio on the recoveries of antioxidants from *C. olitorius* leaves as presented in Figs. 3f and 4f reflected that increasing solvent/sample ratio and reducing ethanol concentration yielded increasing antioxidants. Thus, the optimal predicted antioxidant yields (both DPPH and ABTS assays) were 68.8396 and 29.6220 $\mu\text{g/mL}$, respectively, at the microwave-enhanced extraction conditions of extraction time, microwave power, solvent/sample ratio, and ethanol concentration at 131.37 s, 305.08 W, 11.67 mL/g, and 50%, respectively.

Validations of the generated models

Based on the predicted values from the Design Expert® software, the optimal conditions to achieve phenolic compounds (TPC of 343.327 mg GAE/10 g d.b.) and antioxidants (IC_{50} values of 68.8396 and 29.6220 $\mu\text{g/mL}$ for DPPH and ABTS assays, respectively) from extracts from *C. olitorius* leaves were found at extraction time of 131.37 s, microwave power

of 305.08 W, solvent/sample ratio of 11.67 mL/g, and ethanol concentration of 50% with the desirability of 0.942. The validation experiments were carried out using approximated values of the predicted values such as extraction time of 131 s, microwave power of 305 W, solvent/sample ratio of 12 mL/g, and ethanol concentration of 50%. Three replicate experiments were carried out using these conditions, and the mean was determined to affirm the generated outcomes from the software. The experimental trials amounted to TPC of 343.098 ± 3.05 mg GAE/10 g d.b., IC_{50} values of 68.89 ± 1.08 and 29.76 ± 1.00 $\mu\text{g/mL}$ for DPPH and ABTS assays, respectively, at 131 s, 305 W, 12 mL/g, and 50% of extraction time, microwave power, solvent/sample ratio, and ethanol concentration, respectively. Using Chi-squared analysis, the results obtained indicated no significant difference between the experimental and predicted values from the software.

As earlier reported, some previous studies had evaluated the contents of total phenols and antioxidants in the leaf extract of *C. olitorius*; however, no study was reported on recovering phenolic compounds and antioxidants from this plant through microwave-enhanced technique. A recent finding reported the recoveries of total phenols (0.10–9.81 $\mu\text{GA/g}$), DPPH inhibition (1.4–70.3%) and ABTS inhibition (83–93%) (Obeng et al. 2020). In another recent study, a total antioxidant capacity of 165.66 ± 1.30 mg ascorbic acid/g dry extract was reported in the leaf extract of *C. olitorius* (Ali et al. 2021). The TPC of 2.5–32.0 Mg TE/g was reported in a recent study; it was established that phenolic compounds and antioxidants from this plant are unaffected by cultivation treatments. Moreover, it had been reported that the mode of extraction can alter the phenolic compounds in the *C. olitorius* leaves (Guzzetti et al. 2021). The antioxidant from *C. olitorius* leaves was reported to be $91.65 \pm 0.15\%$ using DPPH inhibition with the maceration extraction technique (Racha et al. 2018). The results from the current study established the importance of using the microwave-enhanced extraction method to recover optimal yields of phenolic compounds and antioxidants from *C. olitorius* leaves. Other than this, this plant can further be used in combating food insecurity because it is rich in phenolic compounds.

LC-QToF-MS analysis of the optimized extract from *C. olitorius* leaves

The use of LC-QToF-MS analysis to characterize phenolic compounds has been a current trend in natural product chemistry; this analysis has proven to be efficient in tentatively identifying phenolic compounds in diverse plant samples (Alara et al. 2018c, 2018b; Tan et al. 2020; Tang et al. 2020). Unifi software processed the data. Thus, the phenolic profile of the leaf extract of *C. olitorius* at optimized

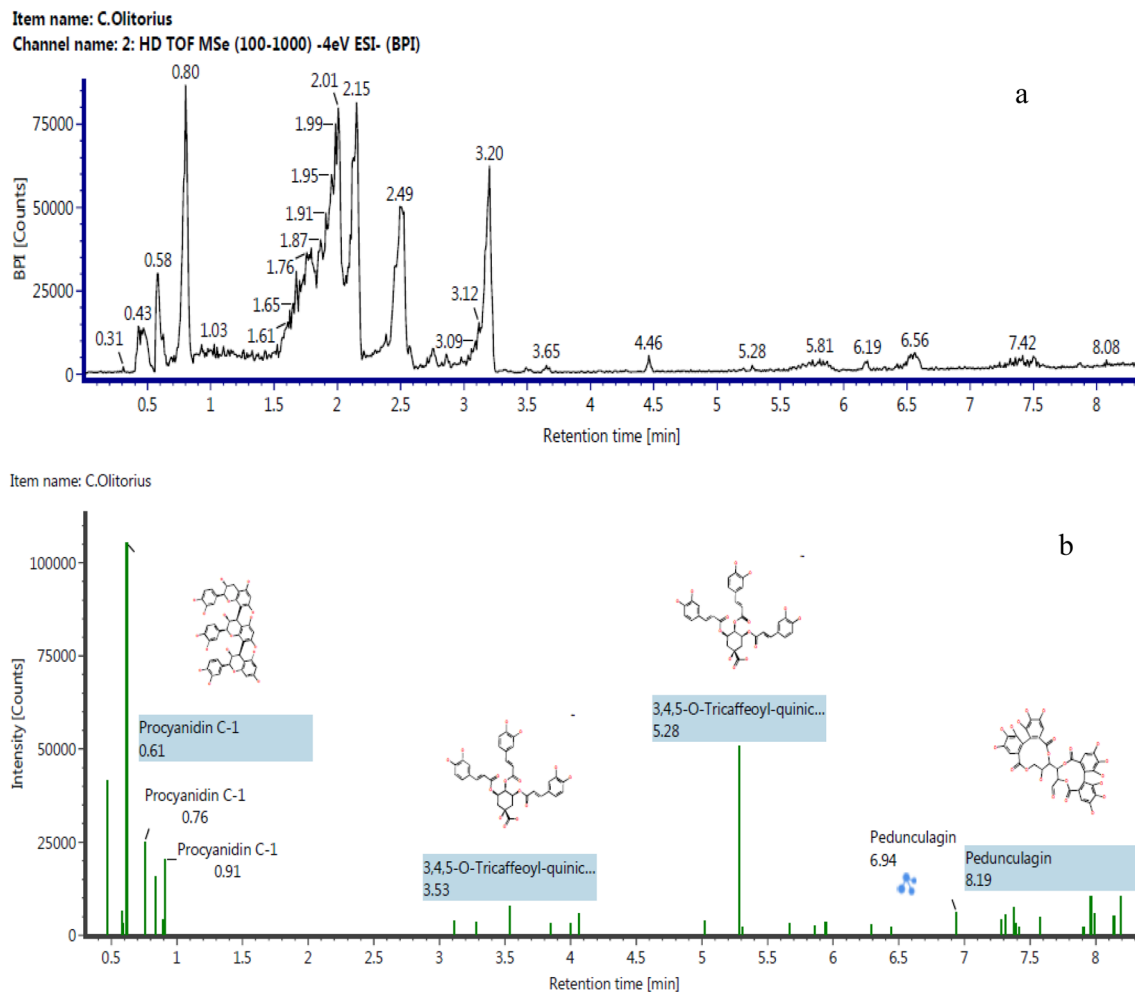


Fig. 5 Negative ionization plots for the identified chemical compounds in the extract from *C. olerius* leaf

microwave-enhanced extraction conditions was characterized using the LC-QToF-MS system (Fig. 5). The characterization and identification of phytochemicals in the leaf extract of *C. olerius* were done by comparing retention time (RT), mass spectrometric (MS), and mass error between mass observed (which must be less than 10 ppm for viable identified compound) (Tang et al. 2020). A total of fourteen phenolic compounds were identified in the extract of *C. olerius* leaves as presented in Table 3. The identified phytochemicals were 1-galloyl-glucose, 1,3,5-O-tricaffeoylquinic acid, procyanidin C-1, 4,4',5,6-tetrahydroxystilbene, 3,4,5-O-tricaffeoylquinic acid, 5-desgalloylstachyurin, sanguini H-4, corilagin_1, 1-O-galloylpedunculagin, laevigatin A, pedunculagin, 2,4,6-tri-O-galloyl- β -D-glucose, 1,3,6-trigalloyl- β -D-glucose, and 1,2,3,6-tetra-O-galloyl- β -D-glucose as presented in the Supplementary materials (Figs. S1–S14).

In simplicity, identified 1-galloyl-glucose, 1-O-galloylpedunculagin, pedunculagin, 2,4,6-tri-O-galloyl- β -D-glucose, 1,3,6-trigalloyl- β -D-glucose, and 1,2,3,6-tetra-O-galloyl- β -D-glucose belong to tannins (naturally occurring

polyphenols). Another identified tannin was 5-desgalloylstachyurin. These compounds are rich in antioxidants and can alleviate different forms of cancer and free radicals (Zhang et al. 2009). Other identified compounds were 1,3,5-O-tricaffeoylquinic acid and 3,4,5-O-tricaffeoylquinic acid; these compounds were reported as polyphenols that are rich in antioxidants (Wang et al. 2017). Procyanidin C-1 belongs to polyflavonoids and bioflavonoids; this compound has been reported natural occurring antioxidant from a plant source (Xu et al. 2021). The identified stilbene monomer in the leaf extract of *C. olerius* was 4,4',5,6-tetrahydroxystilbene. This phytochemical has been reported to possess diverse pharmacological features which antioxidants (Zhang and Chen 2018). Sanguini H-4 had been reported to possess antioxidant properties (Lachowicz et al. 2020). Corilagin_1 and laevigatin A are compounds that are rich in antioxidants as well. Therefore, extract from *C. olerius* leaf has been established as a source of phenolic compounds and natural antioxidants.

Table 3 The tentative assignments of chemical compounds in the leaf extract of *C. olitorius*

S/N	Chemical compound	RT (min)	[M-H] ⁻ (m/z)	Mass error (ppm)	Molecular formula	Response	Adducts
1	1-Galloyl-glucose	0.58	331.0673	0.8	C ₁₃ H ₁₆ O ₁₀	6533	-H
2	1,3,5-O-Tricaffeoylquinic acid	0.59	677.1499	-1.9	C ₃₄ H ₃₀ O ₁₅	3235	-H
3	Procyanidin C-1	0.61	865.1981	0.6	C ₄₅ H ₃₈ O ₁₈	105,272	-H
4	4,4',5,6-Tetrahydroxystilbene	0.75	289.0725	2.5	C ₁₄ H ₁₂ O ₄	6478	-H
5	3,4,5-O-Tricaffeoylquinic acid	3.11	677.1517	0.7	C ₃₄ H ₃₀ O ₁₅	7900	-H
6	5-Desgalloylstachyurin	4.00	783.0674	-1.6	C ₃₄ H ₂₄ O ₂₂	3123	-H
7	Sanguin H-4	5.31	633.0731	-0.4	C ₂₇ H ₂₂ O ₁₈	2263	-H
8	Corilagin_1	5.85	679.0783	-0.8	C ₂₇ H ₂₂ O ₁₈	2547	-H
9	1-O-Galloylpedunculagin	5.95	981.0849	-0.1	C ₄₁ H ₂₈ O ₂₆	3356	-H
10	Laevigatin A	6.47	847.0839	-0.9	C ₃₄ H ₂₆ O ₂₃	2292	-H
11	Pedunculagin	6.94	829.0728	-1.6	C ₃₄ H ₂₄ O ₂₂	6165	-H
12	2,4,6-Tri-O-galloyl-β-D-glucose	7.40	681.0937	-1.4	C ₂₇ H ₂₄ O ₁₈	3128	-H
13	1,3,6-Trigalloyl-β-D-glucose	7.91	681.0954	1.4	C ₂₇ H ₂₄ O ₁₈	2265	-H
14	1,2,3,6-Tetra-O-galloyl-β-D-glucose	8.14	833.1060	0.6	C ₃₄ H ₂₈ O ₂₂	5153	-H

Conclusion

Seeking alternative sources of antioxidants, especially plant-based, will continue to be studied. Given this, this study investigated *C. olitorius* leaves as a source of phenolic compounds and antioxidants by considering a technique that can recover yields (microwave-enhanced extraction) using ethanol/water as extracting solvent. The optimized process established optimal recoveries of phenolic compounds and antioxidants which were higher compared to previously reported results on *C. olitorius* leaves. In addition to this, the phenolic compounds that were comprehensively identified in the extract (fourteen in total) ascertain *C. olitorius* leaves as great sources of antioxidants. This outcome is an indication that *C. olitorius* leaves can continue to be encouraged in daily consumption, a way of combating food insecurity in the world. Thus, future study can focus on isolation and investigation of the tentatively identified phenolic compounds. These can further explain the pharmacological importance of this plant sample.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11696-023-02771-x>.

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Declarations

Conflict of interest We declare none.

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