

## LC–ESI–MS/MS based characterization of phenolic components in fruits of two species of *Solanaceae*

Muhammad Yasir<sup>1,2</sup> · Bushra Sultana<sup>1</sup> · Farooq Anwar<sup>3,4</sup>

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**Abstract** The present work appraises the phenolic profile of fruit extracts from two species (*Solanum indicum* and *Solanum surattense*) of *Solanaceae*. The bioactive components from both the species were extracted with different concentrations of acidified (hydrolysis) and aqueous methanol (non-hydrolyzed) and analyzed for the total phenolics calorimetrically. The hydrolyzed extracts of both the species were characterized for phenolic compounds profiling using LC–ESI–MS/MS. The hydrolysis of fruit samples produced greater extract yield (31.9–50.7%) and total phenolics (250.4–289.5 mg GAE/g of extract) than non-hydrolyzed (11.1–19.2 and 161.5–171.2, respectively). The hydrolyzed extracts from both the species, with higher contents of total phenols were analyzed for phenolic profiling using LC–ESI–MS/MS technique and the data authenticated the presence of caftaric acid, ellagic acid, myricetin, apigenin-7-glucoside and methoxy chrysin in *S. indicum* while isoquercitrin, gallo catechin, quercetin and catechin in *S. surattense*. The current study explores the profile of valuable phenolic bioactives of the selected species of *Solanaceae*, thereby supporting their potential

applications as ingredients of functional foods and nutraceuticals.

**Keywords** Acidified methanol · Hydrolysis · Bioactives extraction · Folin reagent · Phenolic compounds · LC–ESI–MS/MS

### Introduction

Plants are richest sources of a wide array of compounds having antioxidant properties such as phenolics, flavonoids and tocopherols (Ahmad et al. 2016). Plant based natural products enriched with phenolic bioactives and other high-value compounds have been extensively used for many years as folk medicine by traditional physicians to treat several diseases and inflammations (Aktumsek et al. 2013).

The family *Solanaceae* comprising about 98 genera and 2700 species includes numerous important agricultural crops, ornamentals and medicinal plants which are distributed worldwide (Perez et al. 2006). Many plants from this family including annual or perennial herbs, and shrubs or vines are known as potential sources of high-value bioactives. *Solanum* is an important genus comprising of about 1500 species, mostly of medicinal significance (Yadav et al. 2014). Some species (*S. lycopersicum*, *S. melongena*, *S. tuberosum*) are used as staple food while some are highly toxic (Yadav et al. 2016). A number of species has been investigated for their phytochemical constituents and found to contain different bioactive compounds like phenolics, sterols, flavonoids, alkaloids, saponins and glycosides (Premalatha et al. 2013). One of the important species from family *Solanaceae* namely *Solanum surattense* is an important medicinal herb having anticancer and cardioprotective properties. The roots of this

✉ Bushra Sultana  
bushrasultana2005@yahoo.com

<sup>1</sup> Department of Chemistry, University of Agriculture, Faisalabad 38000, Pakistan

<sup>2</sup> Department of Chemistry, University of Sargodha, Faisalabad Campus, Faisalabad 38000, Pakistan

<sup>3</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia

<sup>4</sup> Department of Chemistry, University of Sargodha, Sargodha 40100, Pakistan

herb are beneficial against asthma and cough. The leaves are used to cure rheumatism while the fruits to cure sore throat. Mixture of honey with juice of *S. surattense* fruits and flowers is used for treatment of chronic cough, sore throat, fever, rheumatism and chest pains, bronchitis, colic constipation, dental caries, leprosy, hypertension, cardiac disorder and catarrh (Joseph et al. 2011; Muruhan et al. 2013; Ahmad et al. 2016a). *Solanum indicum* Linn. (known as Gnagan) comparatively less studied specie, is a bushy herb recognized with medicinal effects for the treatment of various diseases. Different parts of this herb such as leaves, fruits and roots are found to be useful in the treatment of appetite loss, blood disorders, cough, sore throat, asthma, sexual disorders, fever, abdominal pain, insomnia and urinary complications etc. On the other hand, Syu et al. (2001) determined the cytotoxicity of this plant and isolated some novel compounds. Typically, the seeds can be applied to cure toothache. Juice from the leaves when mixed with vinegar, can be used as mouthwash. The decoction of roots can be applied as a tonic (N'Dri et al. 2010; Deb et al. 2013).

It is widely accepted that the medicinal health functions of many herbal plants are mainly attributed to the presence of phenolics among others. Until now, the subject species have not been explored for the profile of phenolic bioactives extracted using different solvent systems. The current study therefore was aimed to extract phenolic bioactives from two species namely *S. indicum* and *S. surattense* using different concentrations of aqueous methanol and acidified methanol. The extractable components (both in hydrolysable and non-hydrolysable form) were quantified for total phenols. Moreover, the selected hydrolysable extracts were characterized for the individual phenolic profiling using an advanced LC–ESI–MS/MS approach. Our results are a good contribution to phenolic profiling of medicinal plants data base.

## Materials and methods

### Materials

Samples of *S. indicum* and *S. surattense* were collected from different rural areas of Faisalabad, Pakistan. Fruits of both species were manually separated, washed, shade-dried and ground to fine powders. Shade dried samples were ground, extracted in an orbital shaker (PA 250/25-H) at sample to solvent ratio of 1:10 (w/v) with aqueous methanol (70, 50 and 30% v/v) to get non-hydrolyzed extracts (Zhu et al. 2011). In order to release the bound phenolics, hydrolysis was also performed. In this mode, 10 g of each powdered plant sample was mixed with 100 mL of 0.5, 1.0 and 2.0 M acidified (HCl) methanol and

agitated at room temperature for 24 h (Sroka et al. 2004; Biapa et al. 2007). The hydrolyzed and non-hydrolyzed extracts were freed of excess solvent and concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further analyses.

### Total phenolic contents

Total phenolic contents of the extracts were analyzed using Folin–Ciocalteu reagent according to a reported method of Albano and Miguel (2011) with slight modifications. Each plant sample (0.5 mL having 1 mg dry extract) was mixed with sodium carbonate (2 mL, 7.5%) and Folin–Ciocalteu reagent (2.5 mL, 10%). The mixture was incubated for 30 min at room temperature, then absorbance was recorded at 765 nm using an UV–VIS spectrophotometer (IRMECO, Geesthacht/Germany, Model 5000). Total phenolic contents were quantified based on standard calibration curve for gallic acid (2–200 ppm) and expressed as mg gallic acid equivalents (GAE)/g of dry extract.

### Chemical characterization

The phenolic compounds in 2.0 M acidified methanol hydrolyzed extracts (based upon the higher levels of total phenols) from both the species were further characterized by applying LC/MS technique for the phenolic compounds. Prior to LC/MS analysis, the crude extracts were further purified and concentrated by solid phase extraction (SPE).

### Solid phase extraction (SPE)

Solid phase extraction is a very useful technique to isolate and concentrate phenolic compounds and to remove unwanted and non-phenolic substances. A multichannel SPE cartridge (ThermoFisher Scientific, Waltham, MA, USA) with Strata C-18 columns and vacuum pump was applied to eliminate non-phenolic compounds. Preconditioning of column was done by elution of 1 mL methanol followed by 1 mL distilled water, in order to remove trapped air and to activate ligands present on sorbent surface. Then, 3.5 mL of plant extracts, diluted in methanol/water (50/50), were loaded onto SPE column. Washing was performed with 1 mL of distilled water (2 times) in order to elute all the impurities without affecting sample analytes. To remove all traces of washing solvent and residual matrix, a full vacuum drying was applied for about 5–10 min. In order to recover the phenolic bioactives, elution was carried with three different solvents: 1 mL acetonitrile, then 2 mL methanol and finally 2 mL of 5% formic acid in methanol. All three fractions were combined before subjecting to LC/MS analysis (Naczka and Shahidi 2004; Theerasin and Baker 2009; Tumbas et al. 2007).

### LC–ESI–MS/MS analysis

The phenolic analysis was carried out on liquid chromatography coupled with mass spectrometry (LC/MS) using a ThermoFisher system in which HPLC (Surveyor) system was equipped with linear ESI-Ion Trap (LTQ XL) Mass Spectrometer (ThermoFisher Scientific, San Jose, CA, USA).

In order to get chromatographic separation, 5 µL of each sample was injected through autosampler (Surveyor autosampler plus) into the HPLC system (Surveyor) equipped with reverse phase C-18 column (Phenomenex 250 mm × 4.6 mm, 5 µm particle size). The elution was carried out at a flow rate of 5 mL/min using gradient elution. Solvent A consisted of a mixture of water:acetonitrile:trifluoroacetic acid at 90:10:0.1% (v/v) while solvent B was water:acetonitrile:trifluoroacetic acid at 10:90:0.06% (v/v). Elution was performed using following gradient: 0–10 min: 10–35% B, 10–20 min: 35–42% B and 20–30 min: 42–100% B. A photodiode array was used to detect the compounds and prominent peaks were also analyzed by mass spectrometer (LTQ XL ThermoFisher Scientific). The compounds corresponding to these peaks were ionized using Atmospheric Pressure Electrospray Ionization (ESI) probe at negative ion mode. Other ESI–MS conditions included; sheath gas flow rate 9 L/min, auxiliary gas flow rate 2 L/min, APCI vaporization temperature 300 °C, corona source voltage 4.5 kV, source current 4.10 µA, ion transfer capillary temperature 275 °C, capillary voltage 45 V and tube lens voltage 110 V.

Identification of phenolics was conducted under full scan mode in the range of 100–600 m/z. MS/MS analysis for each parent ion peak was performed at different Collision Induced Dissociation (CID) powers. X-calibur 1.4 software was applied for calibration of MS data (Sun et al. 2007; Kajdzanoska et al. 2010; Imran et al. 2013).

Qualitative analysis was performed by comparing the retention time of identified peaks in HPLC chromatogram with those of reference standards, literature reports and MS spectral library data. Final authentication about chemical nature of a compound came from its MS analysis and fragmentation profile. Quantitative study was carried out by measuring the peak areas of LC chromatograms. Moreover, in MS analysis, the concentration of compounds having their reference standards were directly determined following calibration curve method, while those without reference standards were quantified comparing their MS intensity with those having reference standards using the following relation:

$$C_s = \frac{I_s}{I_o} \times C_o$$

where  $C_s$  and  $C_o$  are the concentrations of compounds without reference standard and having reference standard, respectively while  $I_s$  and  $I_o$  are MS intensities of compounds without reference standard and having reference standard, respectively (Dziri et al. 2012; Rispaïl et al. 2005).

## Results and discussion

### Extraction yield

Extraction is a key step for the separation of bioactive compounds from plant materials and their utilization for natural product developments (Yasir et al. 2016). Table 1 represents extract yields for both species of *Solanaceae* obtained by different extraction solvents. Acidified methanol (2.0 M) extracted maximum amount of extracts from both the species while 30% methanol produced maximum amount of extracts among non-hydrolyzed extraction. Aqueous methanol gave better extract yield for

**Table 1** Extract yields (% age) and total phenolic contents (GAE, mg/g) for non-hydrolyzed and hydrolyzed extracts of *S. indicum* and *S. surattense*

	<i>Solanum indicum</i>		<i>Solanum surattense</i>	
	Extract yield (%) <sup>A</sup>	TPC <sup>B</sup>	Extract yield (%) <sup>A</sup>	TPC <sup>B</sup>
70% Methanol	15.3 ± 0.2 <sup>d</sup>	185.5 ± 2.4 <sup>c</sup>	11.1 ± 0.1 <sup>e</sup>	161.5 ± 1.6 <sup>e</sup>
50% Methanol	17.7 ± 0.3 <sup>d</sup>	190.1 ± 2.2 <sup>b</sup>	12.4 ± 0.1 <sup>e</sup>	166.7 ± 1.9 <sup>e</sup>
30% Methanol	19.2 ± 0.1 <sup>d</sup>	191.2 ± 1.2 <sup>b</sup>	15.7 ± 0.4 <sup>e</sup>	171.2 ± 2.4 <sup>e</sup>
2.0 M Acidified methanol	31.9 ± 0.3 <sup>c</sup>	201.4 ± 3.1 <sup>a</sup>	42.4 ± 0.5 <sup>b</sup>	289.5 ± 2.4 <sup>a</sup>
1.0 M Acidified methanol	41.2 ± 0.1 <sup>b</sup>	200.4 ± 3.2 <sup>a</sup>	48.9 ± 0.1 <sup>b</sup>	261.5 ± 1.6 <sup>b</sup>
0.5 M Acidified methanol	47.3 ± 0.4 <sup>a</sup>	191.5 ± 2.4 <sup>b</sup>	50.7 ± 0.3 <sup>a</sup>	250.4 ± 1.7 <sup>c</sup>

Values are mean ± SD of three replications

Different letters in each column represent significant differences ( $p \leq 0.05$ ) among solvents used

<sup>A</sup> % (w/w dry biomass). <sup>B</sup> TPC, total phenolic contents expressed as mg gallic acid equivalents (GAE)/g extract

*S. indicum* ( $19.2 \pm 0.1\%$ ) than for *S. surattense* ( $15.7 \pm 0.4\%$ ), while acidified methanol showed better yield for *S. surattense* ( $50.7 \pm 0.3\%$ ) than *S. indicum* ( $47.3 \pm 0.4\%$ ). These results can be explained by the differences in solubility of various phytochemicals into the extracting solvents depending upon their chemical nature as well as polarity and nature of extraction media. It seemed that *S. indicum* has more contents of water soluble compounds whereas *S. surattense* contained higher concentration of conjugated (hydrolysable) phenolics.

### Phenolic contents

The total phenolic contents as gallic acid equivalents of six different extracts for both species of *Solanaceae* family are presented in Table 1. For both species, 2.0 M acidified methanol offered maximum phenolic contents ( $201.4 \pm 3.1$  and  $289.5 \pm 2.4$  mg GAE/g dry extract for *S. indicum* and *S. surattense*, respectively) among hydrolyzed extracts. While 30% methanolic extract exhibited higher phenolic contents ( $191.2 \pm 1.2$  and  $171.2 \pm 2.4$  mg GAE/g dry extract for *S. indicum* and *S. surattense*, respectively) among non-hydrolyzed extracts, which are quite comparable with acetone and methanolic extracts of *S. surattense* leaf ( $16.7 \pm 2.5$  and  $16.6 \pm 1.6$  g/100 g extract),

respectively (Joseph et al. 2011). The results of the present study indicated that hydrolyzed extraction was an efficient way to squeeze out free as well as bound phenolic compounds from internal plant cellular organelles.

### LC/MS analysis phenolics

Table 2 shows the results of LC–ESI–MS/MS analysis of phenolics enriched 2.0 M acidified methanolic extracts of *S. indicum* and *S. surattense*. Detailed description of each specie is given below:

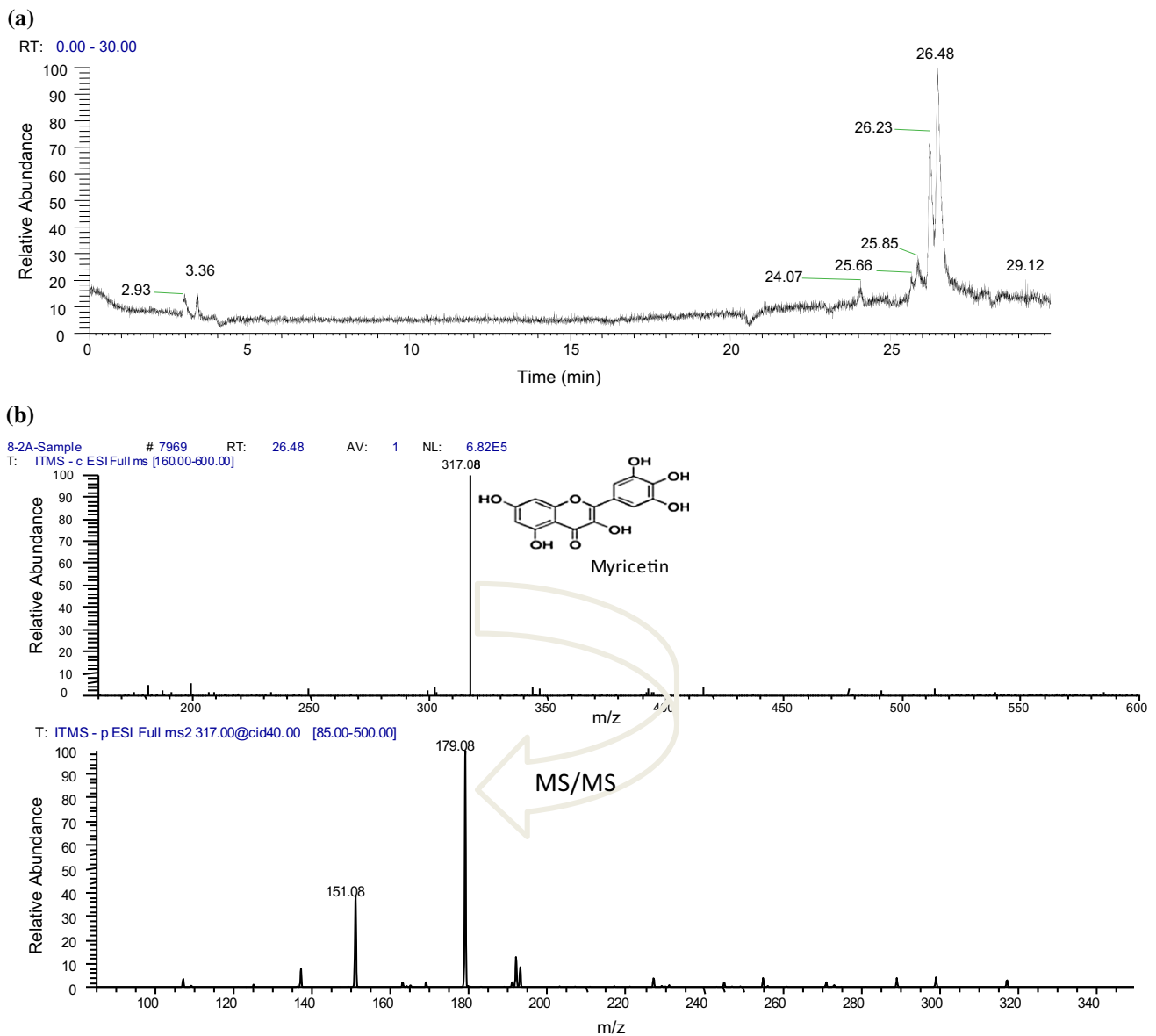
#### *Solanum indicum*

*Phenolic acids and their derivatives* HPLC chromatogram (Fig. 1a), showed eight major peaks five of which were identified and corresponded to different phenolics and flavonoids. Peak 5 (RT 25.85 min) presenting a  $[M-H]^-$  ion at  $m/z$  301.10, gave the indication of ellagic acid. The molecular ion peak produced fragmented peaks at  $m/z$  257 by removal of  $-COO$  group (Seeram et al. 2006). Peak 6 (RT 26.23 min) displayed a  $[M-H]^-$  at  $m/z$  311.10 and was considered to be caftaric acid. The parent ion peak was subjected to fragmentation that gave rise to three daughter ions at  $m/z$  179, 149 and 135. First peak ( $m/z$

**Table 2** LC/MS profile of hydrolyzed extract of *S. indicum* and *S. surattense*

Peak no.	RT (min)	MW	$[M-H]^-$	$MS^2$ ions	Proposed compounds	Molecular formula	Concentration <sup>A</sup>	References
<i>Solanum indicum</i>								
1	2.93	284.26	283.04	268, 239, 211	Methoxy chrysin	$C_{16}H_{12}O_5$	263.36	Keckes et al. 2013
2	3.36	–	297.00	281	Unknown	–	270.64	–
3	24.07	–	187.20	128	Unknown	–	270.29	–
4	25.66	432.38	431.15	269, 268	Apigenin-7-glucoside	$C_{21}H_{20}O_{10}$	289.27	Carazzone et al. 2013
5	25.85	302.20	301.10	257, 229	Ellagic acid	$C_{14}H_6O_8$	303.83	Seeram et al. 2006
6	26.23	312.23	311.10	149, 179	Caftaric acid	$C_{13}H_{12}O_9$	333.52	Carazzone et al. 2013
7	26.48	318.24	317.08	179, 151	Myricetin	$C_{15}H_{10}O_8$	403.39	Standard
8	29.12	–	470.96	411	Unknown	–	269.91	–
<i>Solanum surattense</i>								
1	3.36	–	339.00	295	Unknown	–	258.38	–
2	20.95	–	226.93	168	Unknown	–	260.77	–
3	21.58	290.26	289.02	245, 205, 179	Catechin	$C_{15}H_{14}O_6$	262.31	Sun et al. 2007
4	22.69	464.38	463.16	301	Isoquercetrin	$C_{21}H_{20}O_{12}$	528.57	Sanchez-Rabaneda et al. 2003
5	23.82	306.27	305.11	179, 125	Gallocatechin	$C_{15}H_{14}O_7$	339.34	Sun et al. 2007
6	25.04	–	533.03	501	Unknown	–	264.64	–
7	27.06	302.24	301.03	179, 151	Quercetin	$C_{15}H_{10}O_7$	272.97	Standard

<sup>A</sup> Concentration expressed in  $\mu\text{g/g}$  extract



**Fig. 1** a Typical LC–MS chromatogram of hydrolysed (2.0 M acidified methanol) extract of *Solanum indicum* showing separation of phenolics, b MS spectrum with fragmentation pattern of myricetin

z 179) corresponded to molecular ion of caffeic acid by losing tartaric acid residue, second peak (m/z 149) remained unidentified while third (m/z 135) for decarboxylated caffeic acid (Carazzone et al. 2013). Presence of caffeic acid was also reported by N'Dri et al. (2010) applying LC–MS technique.

**Flavonoids and their derivatives** Peak 1 at RT 2.93 min (Fig. 1a) gave a pseudo molecular ion at m/z 283.04 for methoxy chrysin which fragmented to give three sub peaks. The fragmentation pattern provided proof for methoxy chrysin. First fragment ion at m/z 268 by losing one  $\text{CH}_3$  residue while other two peaks were indication for chrysin as

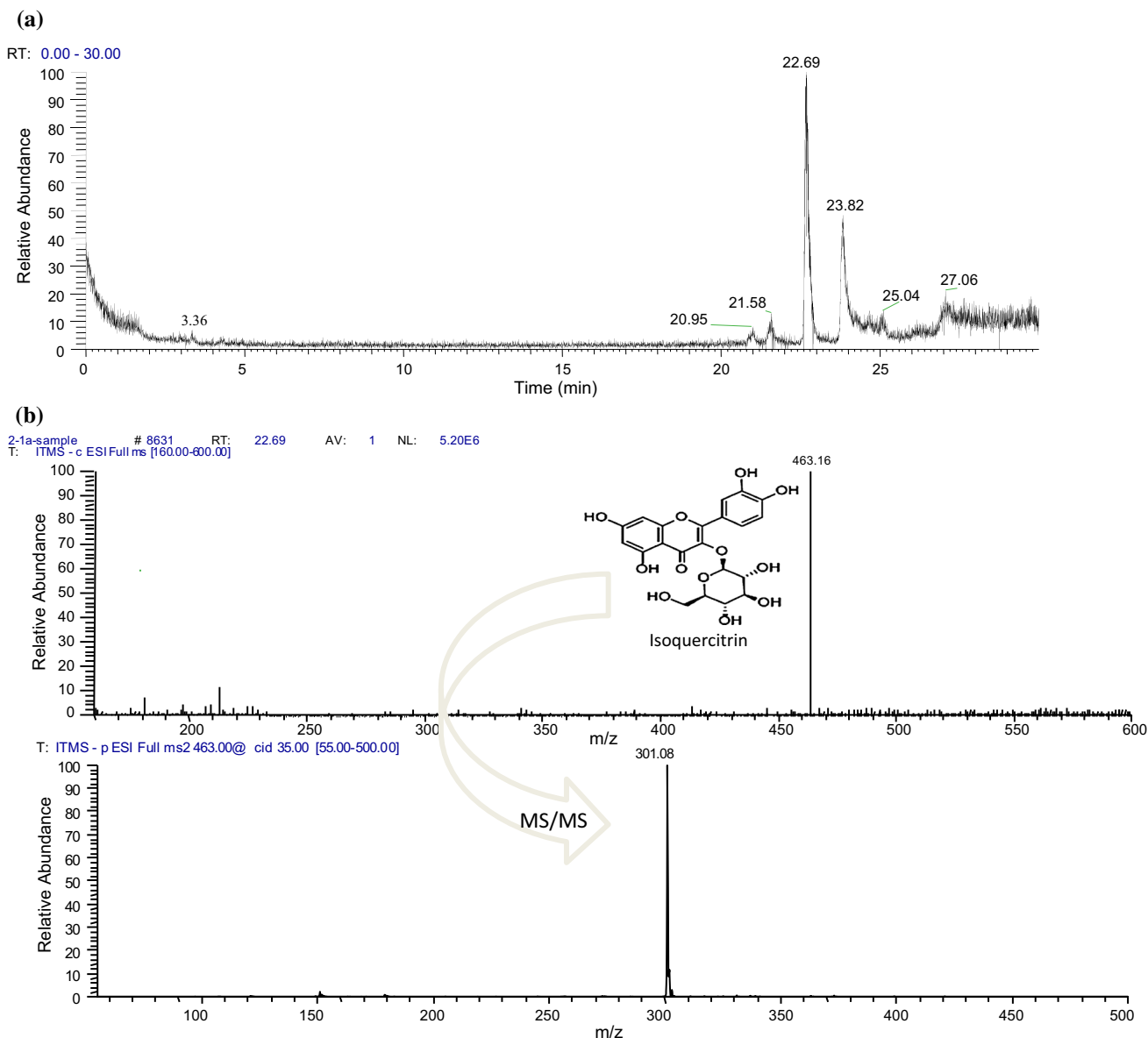
represented by Keckes et al. (2013). Peak 4 (RT 25.66 min) with a molecular ion peak at m/z 431.15 representing apigenin-7-glucoside, yielded daughter ion peak at m/z 269 which was related to apigenin, formed as a result of removal of glucose residue other peak at m/z 268 indicated the presence of glucose residue (Carazzone et al. 2013). Myricetin corresponds to peak 7 (RT 26.48 min) in HPLC chromatogram that yielded an intense molecular ion peak at m/z 317.08. It was further confirmed by two daughter ion peaks at 179 and 151 as presented in Fig. 1b, matched with that of reference standard. Quantitatively, myricetin was found in the highest concentration (403.39 ppm), while all other bioactive compounds were in moderate concentration.

*Solanum surattense*

**Flavonoids and their derivatives** In LC–MS chromatogram, showed seven peaks and four of these were identified as flavonoids and their conjugates as shown in Fig. 2a. Peaks 1 and 2 remain unidentified. Peak 3 of LC chromatogram at RT 21.58 min corresponded to catechin, representing molecular ion at  $m/z$  289.02 which subsequently broken down to give three peaks; one at  $m/z$  245 by losing  $(\text{CH}_2)_2\text{OH}$  group and other peaks at  $m/z$  205 and 179 (Sun et al. 2007). Peak 4 (RT 22.69 min) represented isoquercitrin by a deprotonated molecular ion peak at  $m/z$  463.16. It was then authenticated by matching

fragmentation profile to previously published report of Sanchez-Rabeneda et al. (2003). Fragmentation gave an intense peak at  $m/z$  301 which coincided with quercetin by losing glucose moiety (Fig. 2b).

Peak 5 having retention time 23.82 min, indicated the presence of gallic acid, a flavanol. MS analysis presented a  $[\text{M}-\text{H}]^-$  ion at  $m/z$  305.11 which produced two product ion peaks at  $m/z$  179 (loss of decarboxylated gallic acid residue) and 125 for an ion of decarboxylated gallic acid (Sun et al. 2007). Quercetin was characterized by peak 7 (RT 27.06 min) of LC chromatogram which gave MS peak at  $m/z$  301.03 that fragmented to produce two peaks at  $m/z$  179 and 151 due to Retro-Diels–Alder cleavage of its



**Fig. 2** a Typical LC–MS chromatogram of hydrolysed (2.0 M acidified methanol) extract of *Solanum surattense* showing separation of phenolics, b MS spectrum with fragmentation pattern of isoquercitrin

chemical structure. Final confirmation came from matching MS/MS profile with that of standard. Muruhan et al. (2013) also revealed the presence of flavonoids, terpenoids, tannins and sterols in *S. surattense* but not phenolic acids.

## Conclusion

The current study investigates the extracts yield and total phenols of hydrolyzed and non-hydrolyzed extracts from fruits of the tested species. Hydrolyzed extracts of both the species offered good results as compare to non-hydrolyzed extracts. Moreover, chemical characterization of phenolic bioactives in hydrolyzed extracts of *S. indicum* and *S. surattense* by LC–ESI–MS/MS indicated the presence of myricetin, caftaric acid, ellagic acid, apigenin-7-glucoside and methoxy chrysin in *S. indicum*, while isoquercitrin, galocatechin, quercetin and catechin in *S. surattense*. Thus current study explores the valuable phenolic bioactives profile of the selected species of *Solanaceae*, thereby supporting their potential applications as ingredients of functional foods and nutraceuticals.

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