Evaluation of the phenolic profile, mineral, and fatty acid content and antioxidant activity of Black cumin before and after an in vitro simulated gastrointestinal digestion

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Introduction

Phytochemicals of spices exert promising health benefits due to their antioxidant activities and inhibitory effects against oxidative damage, which is usually involved in several illnesses, including cardiovascular diseases and cancer (Ansary et al., 2020). For this reason, the aim of our study was to evalute locally grown BARI black cumin-1(N. sativa, Ranunculaceae) by (a) characterizing the polyphenolic profiles, (b) evaluating the total phenolic and flavonoid contents, c) determining the lipid profile, d) assessing the antioxidant activities, e) analyzing the mineral profile f) evaluating the gastrointestinal digestion of phenolic compounds to known the mechanisms by which polyphenols are metabolized *in vivo* and exert their healthy activities.

Experimental Methods

Black cumin sample

Samples were extracted according to procedures previously described by Thilakarathne, et al. (2018) with minor modifications (Figure-1)

Figure-1 Method of Black Cumin Extaction and analysis of Phytochemicals

In vitro digestion

This method was modified from that reported by Gil-Izquierdo et al. (2002) with some modifications (Figure 2).

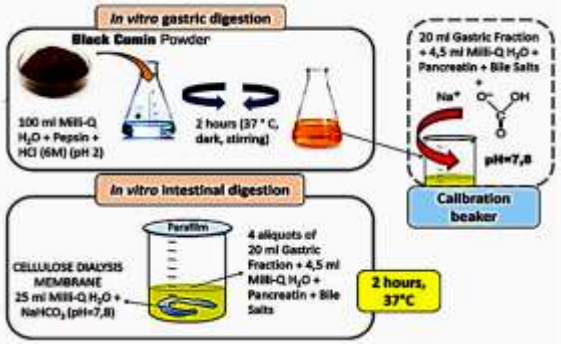

Figure-2 Graphical and schematic representation of the in vitro gastrointestinal digestion.

Determination of Total phenol and flavonoid contents

TPC assay: was assessed through the Folin-Ciocalteu method, as previously described by Singleton et al. (1999).

TFC assay: was measured by the $AlCl_3$ method described by Ariza et al., (2016)

Total antioxidant capacity (TAC)

TAC of the black cumin extract was determined thought the following methods:

TEAC assay: was carried out according to the modified method of Re and co-workers (1999).

FRAP assay: was performed according to the protocol by Benzie and Strain (1996).

DPPH assay: was carried out according to the protocol of Kumaran and Karunakan (2007).

Results

The result obtained from black cumin extract (Total phenolic content and Total flavonoid content) were showed in Table-1.The phenolic compounds identified are reported in Table-3. Our finding confirms that high phenolic components were dihydroxybenzoic acid (3.11 ± 0.083 µg/mg) and ferulic acid (2.06 ± 0.027 µg/mg) respectively. Furthermore, we found 6 different minerals (Ca, Cu, Fe, K, Se, and Zn) in methanolic extracts, as shown in Table 2. Moreover, 25 fatty acids (13 saturated, 7 unsaturated and 5 unsaturated omega fatty acids) were identified, with linoleic acid the most present. In addition, black cumin exhibited strong antioxidant capacity as shown in Table-1. Finally, a significant decreased in the quantity of phenolic compounds (78%) and flavonoids (95%) was found after gastrointestinal digestion in the bioaccessible fraction (In fraction) as well asa reduced the antioxidant activity (38%-79%).

Table-1: Total phenolic content, Total flafonoid contentl and total antioxidant capacity of black cumin.

Parameters	Values
TPC (mg GAeq/g)	3.50 ± 0.17
Flavo (mg CATeq/g)	2.82 ± 0.49
TEAC (µmol Txeq/g)	11.03 ± 1.07
DPPH (µmol Txeq/g)	8.39 ±1.11
FRAP (µmol Txeq/g)	450.17 ± 7.67

Table-2: Mineral composition of black cumin by ICP-MS analysis..

Name of mineral	Results (mg/Kg)
Ca	169.9 ± 3.5
Cu	6.73 ± 0.14
Fe	8.71 ± 0.18
K	3542 ± 120
Se	< 2.97 ± 0.11
Zn	13.36 ± 0.28

n.d. not detected; Results are expressed as mean ± SD (n = 3)

Table-3: Identification of polyphenols and flavonoids in black cumin by HPLC-MS/MS analysis

Compound	Molecular formula	Molecular Mass	Retention Time	Experimental m/z	Fragments detected	Results µg/g
Caffeic acid	$C_9H_8O_4$	179	12.1	178.9	107/135	0.28 ± 0.007
p-coumaric acid	$C_9H_8O_3$	163	13.1	162.9	119/93	0.09 ± 0.003
Dihydroxybenzoic acid	$C_7H_6O_4$	154	8.3	152.9	90.9/109	3.11 ± 0.083
Gallic acid	$C_7H_6O_5$	169	3.7	168.9	124.9/79	1.06 ± 0.080
Salicylic acid	$C_7H_6O_3$	138	14.3	136.9	93.1/64.9	0.11 ± 0.002
Sinaptic acid	$C_{11}H_{12}O_5$	223	13.3	223	163.9/192.9	0.35 ± 0.007
Ferulic acid	$C_{10}H_{10}O_4$	193	13.3	195	176.9/89	2.06 ± 0.027
Quercetin	$C_{15}H_{10}O_7$	302	14.5	301	150.9/221	0.04 ± 0.001

n.d. not detected; Results are expressed as mean ± SD (n = 3)

Discussion

Polyphenols are the most important bioactive compounds present in black cumin.Our results found significant amount of polyphenols in black cumin extract, such as dihydroxybenzoic acid, gallic acid, caffeic acid, and p-coumaric acid that act as strong antioxidants and suppress oxidative stress-related damage and inflammation (Kim et al., 2013). The TAC results also indicated that back cumin has strong antioxidant property. As shown in figure-1, gastric condition was more favour to the greater release of phenol from the black cumin food matrix after gastrointestinal digestion. However, the portion of phenol that could go through the stomach to the intestine during digestion was 17.67% for TPC and 4.33% for TFC. These results demonstrate that some phenolic compounds are absorbed by stomach (Fang., 2014). In accordance, the portion of phenolic compounds potentially gone through the intestine during intestinal digestion (i.e % of bioaccessibility of intestinal fraction) was 32% for TPC and 4.67% of TFC (Figure 2). The less amount of phenolic compound in intestine absorption demonstrated that bioavailability of phenolic and flavonoids depends on many factors such as chemical structure of antioxidant and interaction with other components (Güven. et al., 2010). Accordingly, antioxidant capacity was high in gastric fraction than intestinal fraction shown in figure-3.

Conclusion and Future Aspects

The obtained results showed black cumion as strong antioxidant due to the presence of polyphenols even if half of the phenolics were lost during the digestion process, but significant antioxidant activities were still observed in the digested extracts. Further studies are need to provide more conclusive evidence for clear understanding of the significance of the potential health benefits of the black cumin.

References

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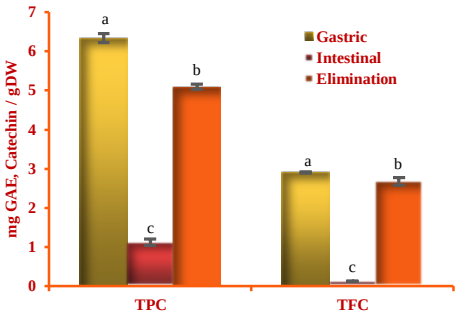

Figure 1: Total phenolic content and flavonoid of cumin after gastric and intestinal digestion. Data were reported as a mean ± SD. Columns labelled with different letters are significantly different ($p<0.05$).

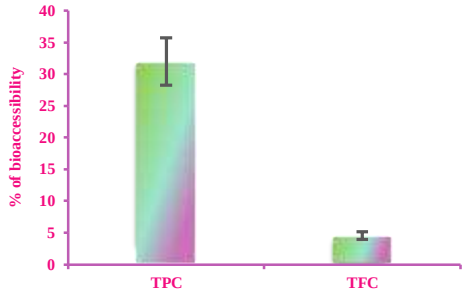

Figure 2: Bioaccessibility percentage of total phenolic and flavonoid black cumin compare to the undigest sample. Data were reported as a mean ± SD.

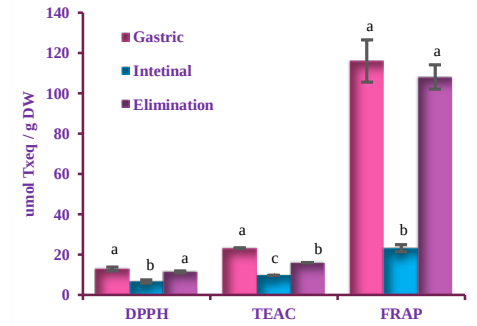

Figure 3: Antioxidant capacity of gastric, intestinal digestion and Elimination fraction of black cumin. Data were reported as mean ± SD. Columns labelled with different letters are significantly different ($p<0.05$).