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ORIGINAL ARTICLE

Evaluation of Antioxidant Activity of Phytochemicals isolated from *Cucurbita pepo L.* seeds

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ABSTRACT
Free radical-induced oxidative stress is a major cause of the progression of many diseases which can be avoided through the exogenous supply of plant-based antioxidants. The purpose of present study is to investigate antioxidant activities of phytochemicals extracted from Cucurbita pepo seeds using 1, 1diphenyl-2-picryl hydrazyl (DPPH), nitric oxide (NO), reduced glutathione (GSH), and total antioxidant capacity (TPC) assays. Total phenol (TPH), total flavonoids (TFL), and total terpenoids (TTE) fractions were found to cause significant free radical scavenging effects as compared to total phytochemicals (TPC), total alkaloid (TAL), andtotalsaponin (TSA) and, their scavenging activity is indicated in IC_{50} values, being 51.3 ± 7.2, 54.9 ± 3.1, 55.4 ± 2.9 µg/mL in TPH, TFL and TTE, respectively. Similarly, inhibition of NO was observed higher in Ascorbic acid (58.1 ± 5.2 µg/mL), TPH (90.9 ± 1.7 µg/mL) and TFL (96.5 ± 7.8 µg/mL). Moreover, the dose-dependent response in the activity of total glutathione S-transferase enzyme was studied and level of GSH was found significantly higher in TPH (81.4 ± 1.5 µg/mL) and TFL (88.3 ± 2.2 µg/mL) at the highest tested concentration (1000 µg/mL). The total antioxidant capacity were found more in total phytochemical (53.4 ± 4.7 mg AAE/ g) followed by TPH (44.6 ± 1.3 mg AAE/ g), TFL (40.3 ± 5.4 mg AAE/ g) and TTE (34.5 ± 4.1 mg AAE/ g). Hence, the present investigation has provided significant information about the antioxidant activities of various isolated fractions of C. pepo seeds and indicated their usefulness in the food industry as a natural source of antioxidant.
KEYWORDS: *Cucurbitapepo L.,Oxidative stress, Antioxidant, Phytochemicals, 1, 1diphenyl-2-picryl hydrazyl, nitric oxide, reduced glutathione, and total antioxidant capacity.*

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INTRODUCTION

Plant-based antioxidants have gained attention and rapid acceptance all over the world[1]. An antioxidant is a substance that is capable of preventing oxidation stress which is caused due to the imbalance between cellular production of free radicals like reactive oxygen species (ROS) and loss of the ability of cells to scavenge them. The abundance of natural antioxidants has been reported in vegetables, herbs, spices, grain, fruits, leaves, roots, and, oilseeds. Due to the presence of a significant amount of phytochemicals, plants have received substantial attention worldwide.

Oxidative stress can be minimized by providing the antioxidant-rich diet supplement [2]. These supplements also boost endogenous antioxidants and help the body to combat the unwanted effects of reactive oxygen species (ROS) induced oxidative damage. Moreover, they also possess inherent ability to produce a wide range of non-enzymatic antioxidants capable of attenuating ROS-induced oxidative damage which suggest that natural bioactive compounds can be used as a safer and effective alternative of a chemical synthetic drug [3, 4]. Free radicals have long been identified as causative agents of many human ailments and several studies have reported the oxidative stress-induced pathology includes cell damage [5, 6],cancer [7,8]cardiovascular disease [9], neural disorders, Alzheimer's disease, Parkinson's disease, mild cognitive impairment [10-14] alcohol-induced liver disease [15], ulcerative colitis [16], atherosclerosis [17], and aging [18].

Cucurbitaceae is a large family comprises of about 130 genera and approximately 800 species, of which genus *Cucurbita* contains three important cultivated species of pumpkin; *C. maxima, C.pepo*, and *C.moschata*[19]. Pumpkin has been used for centuries as a vegetable crop for human consumption and medicinal values. Numerous health benefits of pumpkin seeds have been reported as it contains high levels of bioactive ingredients, including β-carotene, unsaturated fatty acids, phenolic compounds, phytosterols, and tocopherols [20,21]. Additionally, it is also a very good source of the minerals phosphorous, magnesium, manganese, zinc, iron, and copper. Besides seeds, different parts of the pumpkin are edible and have been also used as traditional medicine since antiquity[4, 22-25].

The presence of macro- and micro-constituent *C. pepo* seeds are attributed in providing many health benefits like antiandrogenic, immunological, antiviral, antifungal, cardiovascular, anti-inflammatory, and hepatoprotective activity[26,27]. The functional food ingredients have been reported to possess both anti-inflammatory and antioxidant activities like pumpkin fruit, flower, and seeds oil crude extract. Also, the antioxidant potential of the *C. pepo* seeds in the methanolic, chloroform, and ethyl acetate extracts has been reported by the few researchers[28-29].

However, diverse classes of phytochemicals are reported in *C. pepo* such as alkaloids, flavonoids, phenols, saponins, and, terpenoids that might be responsible for providing therapeutic benefits, but a limited study on the antioxidant potential of isolated phytochemicals has been conducted till now. Therefore, the objective of the present study was aimed to characterize the phytochemical fractions of pumpkin seeds and evaluate the antioxidant properties of isolated phytochemicals of *C. pepo* seeds to apply the existing knowledge in drug discovery and development.

MATERIAL AND METHODS

Extraction of phytochemicals from *C. pepo* seeds

*C. pepo*seedswere obtained from the local market (Orgrain India, India: FSSAI LIC No.12218009000371) then rinsed in double-distilled water, followed by a drying and grinding procedure using an electric mixer. Isolation of phytochemicals viz., alkaloid [30], flavonoid [31], phenol [32], saponin[33], and terpenoid [34]from *C. pepo*were performed using authentic method after defatting of seeds.

Measurement of Antioxidant Activity

Antioxidant activity of various phytochemical isolated from *C. pepo* seeds were analysed by using authentic and established methods of DPPH radical scavenging activity, Nitric Oxide Radical Scavenging Assay, Reduced Glutathione Assay, Total Antioxidant Capacity. [35-40] .

RESULTS AND DISCUSSION

Antioxidants are vital substances which possess the ability to protect the human body from free radicals which can cause oxidative stress, a causative factor for inducing many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, aging, diabetes mellitus, cancer, immunosuppressant, neurodegenerative diseases [41]. A variety of bioactive components of plants including phenolics, tannins, steroids, triterpenoids have been reported as radical scavengers and inhibitors of lipid peroxidation [35]. In the present investigation the antioxidant property of phytochemicals like alkaloid, flavonoid, phenol, saponin, and terpenoid isolated from *C. pepo*seeds was estimated using 1, 1-diphenyl-2-picrylhydrazyl–radical scavenging assay, nitric oxide scavenging assay, estimation of reduced glutathione and total antioxidant capacity.

DPPH free radical method has broadly been employed for the evaluation of the free radical scavenging activity of the natural antioxidants [42]. This analysis is deliberated as one of the most apposite and simple techniques for estimating the free radical scavenging effects of pure compounds from different biological sources [43]. In DPPH analysis, Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical characteristic and generate a reduced state of the DPPH that lead to the disappearance of colour and shows the antioxidant property of the test sample [44, 45]. In our finding, IC50 were found 403.3 ± 4.2, 67.8 ± 6.3, 54.9 ± 3.1, 51.3 ± 7.2, 398.2 ± 9.2, 55.4 ± 2.9µg/mL (Table:1) of total phytochemicals, total alkaloid, total flavonoid, total phenol, total saponin, and total terpenoid respectively isolated from seeds of *C. pepo* whereas IC50 of ascorbic acid used as a standard was observed as 31.1 ± 2.8 µg/mL. Our result revealed that the inhibitory percentage of isolated phytochemical follow a dose-dependent pattern *i.e*. increase in scavenging activity with an increase in phytochemical concentration as well as the inhibitory activity were found nearto the ascorbic acid used as standard. This investigation is in agreement with the study that revealed that crude extract of *Cucurbita moschata* seeds prepared in ethanol-water has IC_{50} value of 435 µg/mL[46]. Similarly, alkaloids isolated from an extract of the stem bark of *Cryptocary anigra* showed IC_{50} values 29.56 µg/mL and 54.53 µg/mL that showed a slight difference with our findings[47]. The bioactive component in a plant like a flavonoid

and phenol showed multiple biological functions and also reported as biologically active antioxidants [48]. Our findings revealed that total flavonoids have IC_{50}at the concentration 54.9 ± 3.1 µg/mL whereas other studies revealed $IC_{50}$of *Pteriscretica L*and *Atriplex laciniata L* at 74.49 µg/mL and 33µg/mL concentration respectively which is slightly different from our investigation [49,50].The antioxidant property of some phenol like quercetin, quercetin hydrate, rutin, catechin hydrate, and kaempferol demonstrated the concentration-dependent antioxidant activity that corroborates with our analysis that is also showing a dose-dependent pattern of free radical scavenging activity [51].It is reported that saponins extracted from *Atriplexlaciniata L*. haveIC_{50}83.37 µg/mL that disclosed a significant difference with our results whereas saponins extracted from the tuber of *Chlorophytum borivilianum* show IC_{50} at a high dose which is concurrent with our result that indicates more concentration of total saponins will be required to scavenge 50% of the free radicals than other phytochemical [50,52] .In our research terpenoid showed IC_{50} at 55.4 ± 2.9 µg/mL concentration which specify that terpenoid isolated from *C. pepo* seeds has high quenching capacity and also several studies already reported that terpenoid extracted from other plant extract has potential antioxidant property [22, 53]. (Figure: 1)

Nitric oxide is classified as a free radical on the basis of unpaired electron and derives from the interaction of NO with oxygen and other free radicals such as superoxide [54, 55]. When reacted with superoxide in the epithelium, it may lead to blood pressure and oxidative damage of DNA due to the formation of peroxynitrite that tends to mutilate a supercoiled DNA structure [56, 57].Under normal physiological conditions, NO acts as a necessary component in the regulation of various functions such as blood pressure, immune response, and neural communication [58]. Overproduction and chronic exposure of nitric oxide radicals can induce tissue damage as well as associated with various carcinomas, hypertension, and inflammatory disease conditions [59, 60]. Therefore, researchers have paid more attention to finding natural antioxidants that may act as potent inhibitors of NO production in relation to the treatment of chronic inflammatory diseases [59]. Our findings also unveiled NO scavenging activity of phytochemicals isolated from *C pepo* seeds when compared with ascorbic acid. The IC_{50} of total phytochemical, total alkaloid, total flavonoid, total phenol, total saponins, and total terpenoids were found 235 ± 3.3, 124.2 ± 8.2, 96.5 ± 7.8, 90.9 ± 1.7, 220.3 ± 3.8, 135.1 ± 4.4 µg/mL(Table:1) whereas in the case of ascorbic acid it was found at 58.1 ± 5.2 µg/mL. Our results are in the agreement with Nafiu et al who reported that saponin extract from the root of *D. basuticus*show the noteworthy inhibitory effect of NO radical[61]. Similar studies also reported in the case of flavonoid (Lakhanpal et al 2007) and terpenoid[22] and they were demonstrated to display an appropriately better NO scavenging strength (Figure: 2)

Glutathione (GSH) plays a significant role in oxidative stress as well as detoxification of xenobiotic [62, 63]. GSH plays many roles in diverse aspects of plant processes including cell differentiation, cell death, enzymatic regulation, senescence, and growth and development, its role as an antioxidant in cellular protection under stress is the most significant one [64]. The results from our study demonstrated that reduced glutathione content was found effective with increasing concentration among all the phytochemicals flavonoids and phenol showed a high level of reduced glutathione 88.3± 2.2 and 81.4± 1.5 µg/mL at 1000 µg/mL of total flavonoid and phenol extract whereas on the same concentration of extract total phytochemical, total alkaloid, total saponin, total terpenoid showed 71.4± 0.64, 75.2± 4.2, 67.5 ± 1.3, 74.6 ± 1.7 µg/mL. It is also reported that simultaneous supplementation with quercetin restored GSH content after oxidative damage in cultured spermatogonial cells [65]. Several other studies also reported the flavonoids could stimulate the transcription of the genes that are responsible for intracellular GSH synthesis [66,67]. Previously, it was also demonstrated that phenolic compounds are found to be inducers of GSH. Gallic acid isolated from Tridham was reported to augment GSH levels. Our finding suggests phytochemical isolated from *C. pepo* may have the potential to stimulate the synthesis of GSH that will help to curtail the oxidative stress (Figure: 3).

The total antioxidant capacity can be measured by a calorimetric quantitative method which measures the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of a bluish-green colored Phosphate-Mo (V) complex. It helps to examine the reduction rate among the antioxidant and molybdenum ligand. This assay is known to analyse to the antioxidant potential of herbal extract [39, 68]. Our results show the total antioxidant capacity of phytochemicals extracted from *C. pepo* seeds more in total phytochemical (53.4 ± 4.7 mg AAE/ g) followed by TPH (44.6 ± 1.3 mg AAE/ g), TFL (40.3 ± 5.4 mg AAE/ g), TTE (34.5±4.1 mg AAE/ g), TAL (21.4 ± 5.9 mg AAE/ g) and TSA (17.3 ± 4.2 mg AAE/ g).

It is therefore concluded that, the analysis of antioxidant reveals that phytochemicals extracted from *C. pepo* seeds may have the potential to reduce oxidative stress and free radical. It can safely be suggested as a potential natural source of antioxidants for utilization in nutritional as well as pharmaceutical fields.

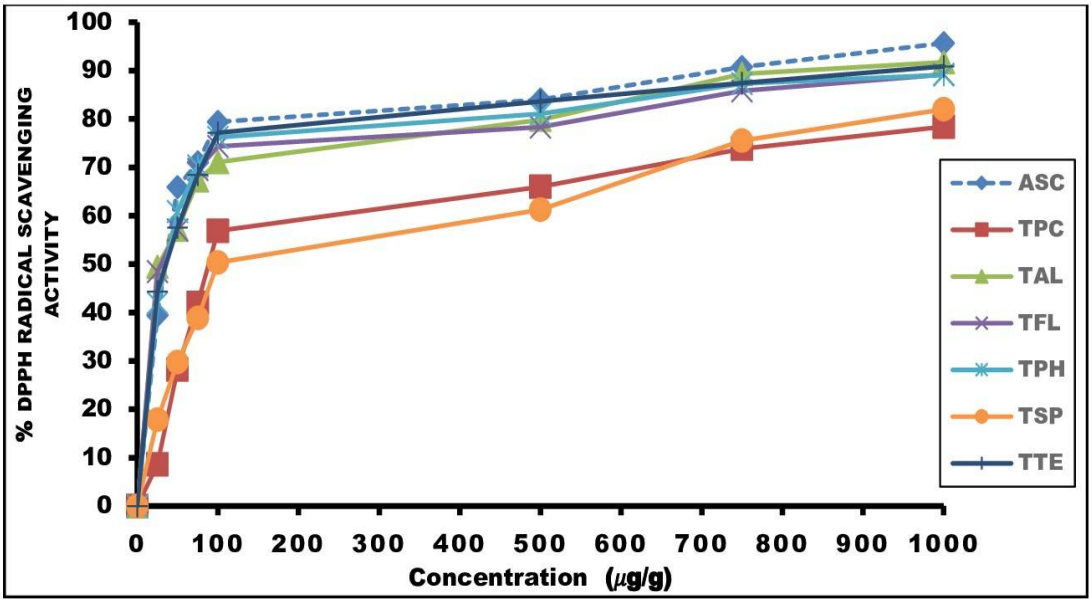

Figure 1: DPPH scavenging activity of phytochemicals isolated from seeds of *C.pepo.* Where ASC: Ascorbic Acid; TPC: Total phytoconstituent; TAL: Total alkaloids; TFL: Total Flavonoid; TPH: Total Phenol; TSA: Total Saponin; TTE: total terpenoid.

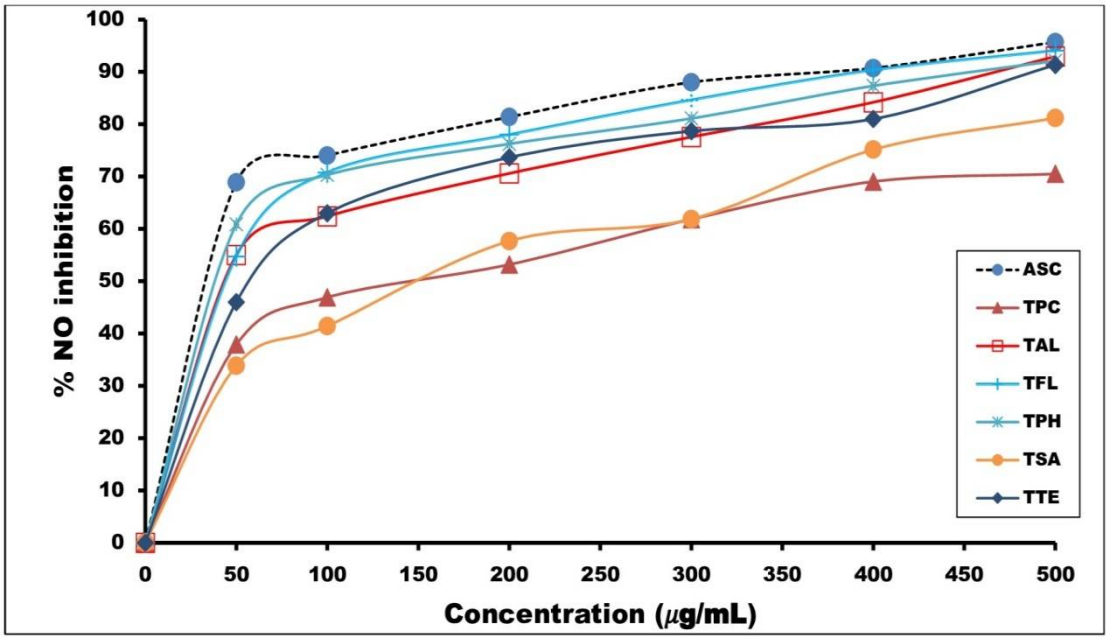

Figure 2: Nitric oxide scavenging activity of phytochemicals isolated from seeds of C. pepo (where ASC: Ascorbic Acid; TPC: Total phytochemical ; TAL: Total alkaloids; TFL: Total Flavonoid; TPH: Total Phenol; TSA: Total Saponin; TTE: Total terpenoid).

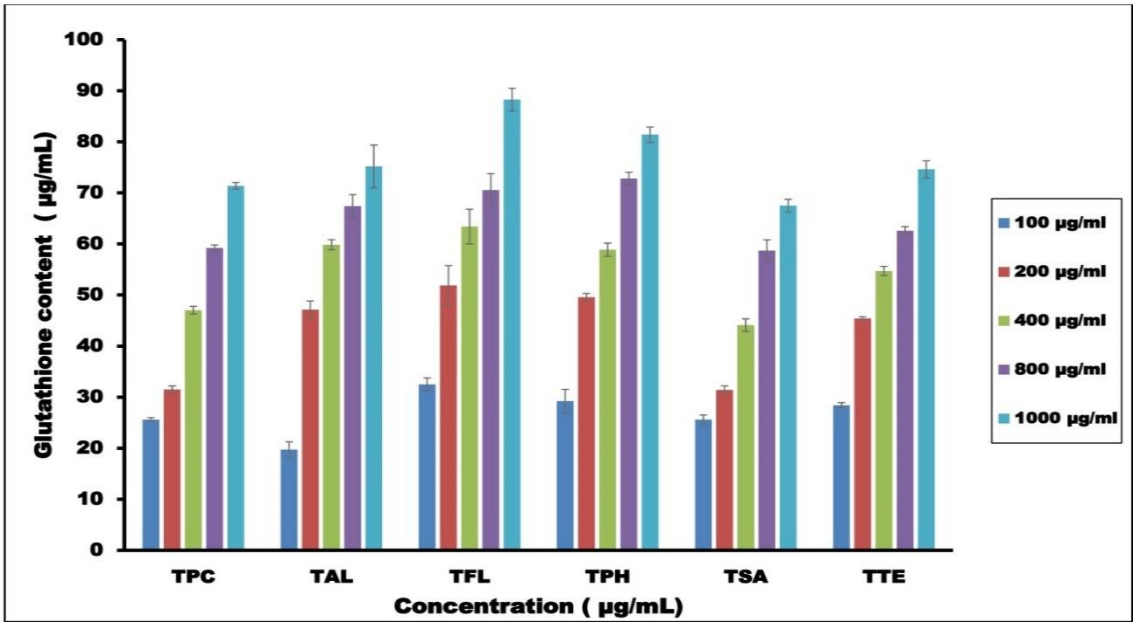

Figure 3: Estimation of reduced glutathione content in the phytochemicals isolated from seeds of C. pepo (where TPC: Total phytochemical; TAL: Total alkaloids; TFL: Total Flavonoid; TPH: Total Phenol; TSA: Total Saponin; TTE: total terpenoid).

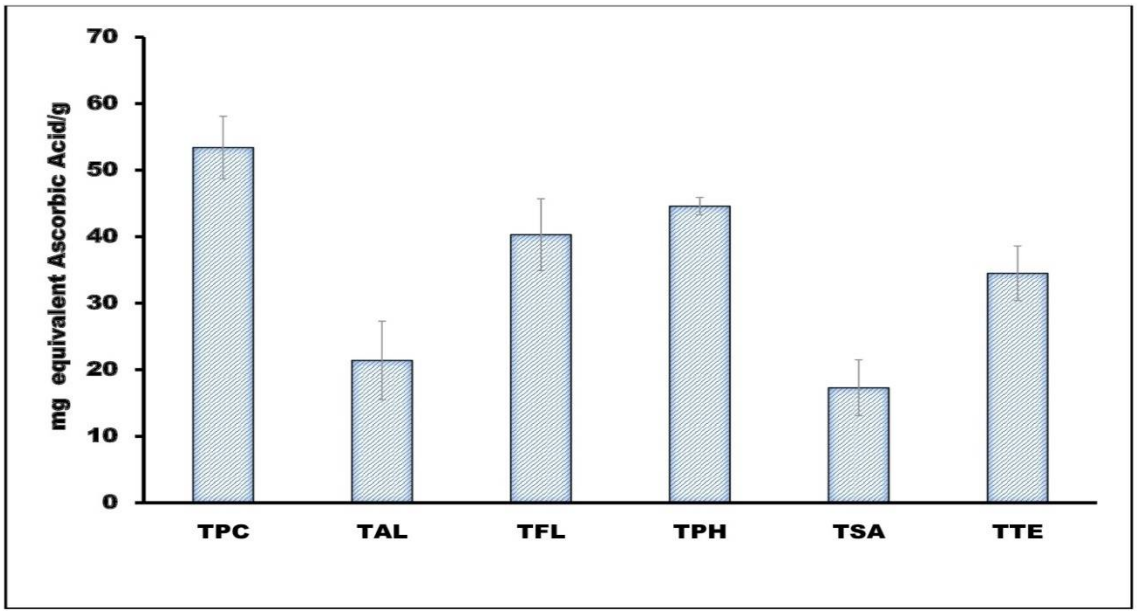

Figure 4: Assessment of total antioxidant capacity of phytochemical isolated from *C. pepo* seeds (where TPC: Total phytochemical; TAL: Total alkaloids; TFL: Total Flavonoid; TPH: Total Phenol; TSA: Total Saponin; TTE: total terpenoid).

Table 1: IC_{50} concentration of phytochemical isolated from C. pepo seeds(Each value is an average of 3 independent replicates)

Phytochemicals	IC_{50} DPPH (µg/mL)	IC_{50} NO (µg/mL)
Ascorbic acid (ASC)	31.1 ± 2.8	58.1 ± 5.2
Total Phytochemical (TPC)	403.3 ± 4.2	235 ± 3.3
Total Alkaloid (TAL)	67.8 ± 6.3	124.2 ± 8.2
Total Flavonoid (TFL)	54.9 ± 3.1	96.5 ± 7.8
Total Phenol (TPH)	51.3 ± 7.2	90.9 ± 1.7
Total Saponin (TSA)	398.2 ± 9.2	220.3 ± 3.8
Total Terpenoid (TTE)	55.4 ± 2.9	135.1 ± 4.4

CONCLUSION

The present investigation has provided significant information and suggests that phytochemical present in the *C. pepo* seeds extract have the potential role in scavenging the free radical.The scientific data validate the antioxidant potential of phytochemicals like alkaloids, flavonoids, phenols, saponin, and terpenoids isolated from*C. pepo* seeds based on the results obtained from DPPH, NO, GSH, and total antioxidant capacity assays. The overall antioxidant activity of *C. pepo* might be attributed to phytochemicals constituents. The findings ofthe current study indicated that seeds of *C. pepo* could be used as a vital source of natural antioxidants and would have used as therapeutic agents in preventing or slowing the progress of reactive oxygen species (ROS) and oxidative stress associated related degenerative diseases.

DECLARATION OF INTERESTS

We declare that no competing interests exist.

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