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Original article

Chromatographic evaluation of gallic acid, catechin and quercetin in methanolic extracts of selected formulations of spices and herbs

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Summary. The main objective of this study was to quantify the probable antioxidants such as gallic acid (phenolic acid), catechin and quercetin (flavonoids) in the three different formulations of mixed spices and herbs, that is mixed herbs (F1), mixed spices (F2) and mixed spices & herbs (F3) predominantly formulated for general health and wellbeing. The aforementioned antioxidants could be highly beneficial for the maintenance of good health as they possess the capacity to scavenge the free radicals which sequentially prevents certain non-communicable diseases (NCDs) such as diabetes mellitus, cancer and atherosclerosis for which free radicals are considered one the major causes. The formulation F2 comprising of mixed spices showed highest contents of gallic acid (86.03 μg/ml), catechin (339.40 μg/ml) and quercetin (394.59 μg/ml) thus highlighting its nutritional potential against various NCDs. The findings of this study depict that the daily supplementation of spices and herbs particularly that of mixed spices could be highly beneficial for the maintenance of good health as they are a rich source of phenolic antioxidants.

Key words: spices, herbs, gallic acid, catechins, quercetin, High Performance Liquid Chromatography (HPLC)

Introduction

With the rapid industrialization and change in lifestyles a high prevalence of certain non-communicable diseases (NCDs) such as diabetes, dementia, atherosclerosis and cancer is being observed nowadays (1, 2). Free radicals that are generated as a result of various metabolic reactions taking place in the body interact with cellular deoxyribonucleic acid (DNA) due to which they are considered one of the major causative factors for NCDs (3-5). To combat the oxidative stress generated by the free radicals the body is naturally equipped with certain endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase (6-8). Along with these natural antioxidants a proper dietary intake of exogenous antioxidants is necessary as the endogenous antioxidants alone could not control the ever challenging oxidative stress (9, 10). This concept strengthens the need of discovering the food sources that contain a high proportion of essential antioxidants (11, 12). Spices and herbs that are traditionally used in various cuisines for preparing and flavoring the food worldwide are equally significant in their medicinal perspective (13, 14). Medicinal and nutritional importance of spices and herbs is based on the probability of the occurrence of a variety of polyphenolic

antioxidants (15, 16). Phenolic acids and flavonoids are the major classes of polyphenols (antioxidants) found in spices and herbs (17, 18). Gallic acid a phenolic acid antioxidant stimulates the production of insulin to control hyperglycemia in type II diabetes mellitus that indirectly protects the pancreatic β-cells from further destruction (19). Quercetin a flavonoid imparts significant anticancer properties by protecting the normal cells and preventing the apoptosis of cancer cells through its antioxidant manifestations (20). Catechin belonging to the flavonoid class of polyphenolic antioxidants is one of the most potent antioxidants being able to protect the β- cells of pancreatic islets thus decelerating the incidence of diabetes mellitus (21). This study has focused on the chromatographic detection and quantification of gallic acid, catechin and quercetin in the methanolic extracts of three formulations of spices and herbs in order to assess their quality in the light of their antioxidant potential.

Materials and Methods

Four spices namely ginger (*Zingiber officinale*), onion (*Allium cepa*), cloves (*Syzygium aromaticum*) and lemongrass (*Cymbopogon citrates*) and four herbs namely coriander leaves (*Coriadrum sativum*), bunching onion (*Allium fistulosum*), curry leaves (*Murraya koenigii*) and holy basil leaves (*Ocimum tenuiflorum*) were used in this study.

The spices and herbs used in this study were purchased from local market. The edible parts were selected and the rest was discarded. The edible parts were washed with distilled water, air dried and then freeze dried. The freeze dried spices and herbs were then ground to fine powder which was than homogeneously mixed to get three formulations of spices and herbs. The first formulation F1 comprised of mixed herbs that is 25% by weight each of powdered curry leaves, holy basil, coriander and bunching onion, second formulation F2 comprised of mixed spices that is 25% by weight each of powdered onion, ginger, clove and lemongrass while the third formulation F3 was the mixture of above two formulations consisting of 12.5% by weight powder of each of the eight spices and herbs used in the study (22, 23).

All the three formulations of spices and herbs were separately rendered to an extraction process. In the first step 1g of the powdered sample was poured in 100 ml of methanol and was sonicated in ultrasonic bath (ultrasound extraction) for 30minutes at the frequency of 60 Hz. In the second step, agitation extraction was performed. For this purpose magnetic stirrer set at 200 rpm speed was used and the sample was stirred for 4 hours to extract the desired antioxidants. The extracts were filtered through Whatman Ashless Filter Paper No.42 and the filtrates were stored at 4°C for further use (24, 25).

Standard solutions of gallic acid (0.5%), catechin (0.5%) and quercetin (0.5%) were prepared separately in methanol and then 1 ml of each solution was transferred into 10 ml volumetric flask and the final volume was made up with mobile phase (30% of 0.1% formic acid : 70% of acetonitrile) to get the final stock solution containing all the three standards. 5ml of the methanolic extracts of each of the formulation of spices and herbs were poured separately in 10ml volumetric flask and the final volume was made up with mobile phase. Before injection into the HPLC system both standard and sample solutions were filtered through 0.22 μm membrane filter (26).

The HPLC system equipped with Photodiode Array (PDA) detector was used for the detection of gallic acid, quercetin and catechins. The wavelength for detection was 280 nm. Kinetex reversed phase C18 silica gel column having the dimensions of 150 mm x 4.6 mm with particle size of 5μm and the pore size of 100Å was used as stationary phase and the column temperature was set at 25°C. The pressure limit was 0 to 6000 psi with the sample run time of 35 minutes. 2.0 μl was the injection volume that was injected into the HPLC system so as to analyze both standard and the samples (27). The system was set at gradient elution program with flow rate of both HPLC pumps A and B was set at 0.8 ml/ minute (27). All the quantitative calculations were made by using the response factor (RF) and the area under curve (AUC) of the detected peaks. The method was validated for the suitability of the system pertaining to the focused antioxidants such as gallic acid, quercetin and catechins for which the repeated tests for validating the retention time and area under curve for each antioxidant were

performed (28). The other validation parameters for the method used for the evaluation of the said antioxidants were tests to validate the precision, linearity and accuracy of peak areas of each antioxidant. Moreover the selectivity of the method used here was validated by comparing the peaks of standard solution , sample solution and blank solution with no interference was observed at any stage (29).

Results and Discussion

The detection of the desired antioxidants was done by comparing the peaks of samples with that of standards on the basis of their retention time (RT) (26).

Figure 1 shows the chromatogram of standard stock solution with peak 1 represents gallic acid, peak 2 represents catechin and the peak 3 represents quercetin.

Figure 2 shows the chromatogram of formulation F1 (mixed herbs) with peak 1 represents gallic acid, peak 2 represents quercetin.

Figure 3 shows the chromatogram of formulation F2 (mixed spices) with peak 1 represents gallic acid, peak 2 represents catechin, peak 3 represents quercetin.

Figure 4 shows the chromatogram of formulation F3 (mixed spices & herbs) with peak1 represents gallic acid, peak 2 represents catechin, peak3 represents quercetin.

As described in Table 1, amongst the tested formulations, F1 showed the presence of gallic acid and quercetin but catechin was not detected in this formulation. The formulations F2 and F3 showed the presence of all the three antioxidants being focused in this study. The formulation F2 (mixed spices) showed the highest contents of the tested antioxidants with gallic acid being 86.03 μg/ml, catechin being 339.40 μg/ml and quercetin being 394.59 μg/ml. The formulation F3 also showed high concentrations of gallic acid (71.48 μg/ml), catechin (129.71 μg/ml) and quercetin (153.44 μg/ml). The quantitative findings of antioxidants in formulations F2 and F3 are highly comparable to the results of a similar study performed by Shan et al., 2005 quantifying the same set of antioxidants in the spices collected from 12 botanical families (13).

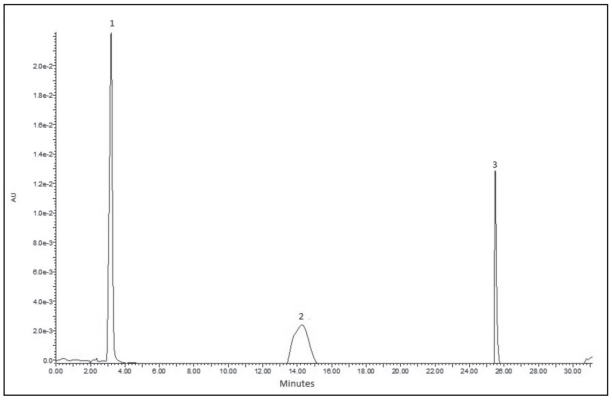

Figure 1. Chromatogram of Standard (gallic acid, catechin & quercetin).

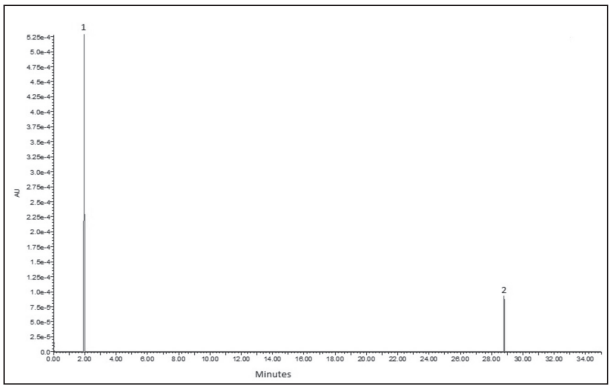

Figure 2. Chromatogram of formulation F1 (mixed herbs).

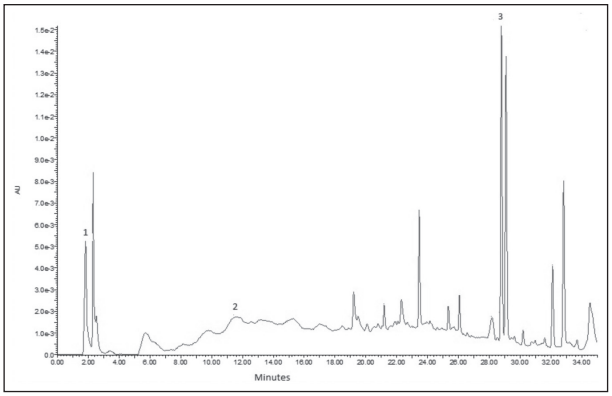

Figure 3. Chromatogram of formulation F2 (mixed spices).

The results of another study performed by Lu and his colleagues using the same chromatographic technique in order to evaluate the phenolic and flavonoid contents of common spices being consumed in China validate the findings of this study under discussion (30). Gallic acid being quantified in all 3 tested extracts of

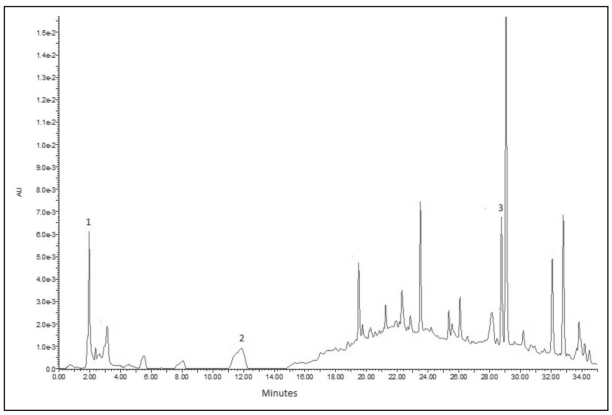

Figure 4. Chromatogram of formulation F3 (mixed spices & herbs).

Table 1. Chromatographic evaluation of gallic acid, catechin and quercetin in the methanolic extracts of tested formulations (µg/ml)

Phenolic antioxidants	Formulation F1 (mixed herbs)	Formulation F2 (mixed spices)	Formulation F3 (mixed spices & herbs)
Gallic acid	15.01	86.03	71.48
Catechin	ND	339.40	129.71
Quercetin	94.30	394.59	153.44

ND= not detected

spices and herbs is a potent antioxidant as described by a study performed on antioxidant contents of Kika fish oil describing a high antioxidant activity of Gallic acid (IC_{50} =29.5µM) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (31). A study describes the FRAP value of 12.5µM of quercetin when the quercetin derivatives from apple, grape and strawberry extracts were tested by Ferric reducing antioxidant power (FRAP) assay (32). A study performed to evaluate the antioxidant power of various catechins described high antioxidant activity of epigallocatechin (EGC) as IC_{50} = 190.6 µM (33). The formulation F2 (mixed spices) showing the highest contents of all the 3 antioxidants being evaluated by HPLC showed a significantly ($P<0.05$) higher FRAP value of 98.9 µg TE/ml compared to the other two formulations when tested for antioxidant activities during this research project. When these formulations were tested by using DPPH the methanolic extract of F3 (mixed spices & herbs) showed the highest activity with the IC_{50} = 65 µg GAE/ml. A similar study performed in Bangladesh focusing on the food preparations mainly comprising of *Murraya koenigii* (curry leaves) described a total phenolic content (TPC) of 17.5 mgGAE/g and a total flavonoid content (TFC) of 16.5 mgCatE/g in the methanolic extracts with the IC_{50} = 24.6 µg/ml for DPPH (34). As the spices & herbs used to prepare the three formulations are important for culinary purposes thus to ascertain the temperature impact on their phenolic and flavonoid contents they were studied in this project for this aspect as well. Amongst the tested cold and hot water extracts of these formulations the cold water extract of F2 (mixed spices) showed highest TPC (2.04 mgGAE/ml) while the hot water (95°C) extract of the same formulation showed a much lower TPC (1.41 mgGAE/ml). This observation depicts that the temperatures have an impact on the phenolic contents of these spices and herbs. Pertaining to the TFC the hot water extract of F1 (mixed herbs) showed highest TFC (0.40 mgQE/ml) while the cold water extract of the same formulation showed TFC (0.22 mg QE/ml). A study performed on the hot and cold water extracts of hibiscus flower, cocoa and ginger beverage has described the relationship of temperatures and time of infusion with the antioxidant contents of the extracts (35). The effect of a wide range of elevated temperatures on the antioxidant contents of the extracts of culinary herbs (yet effective medicinally as well) such as rosemary, oregano and marjoram has been described in a similar study performed by Hossain and coworkers (36). However further research is required to evaluate the impact of temperatures, mode of extractions, modes of drying and other steps of processing of these raw spices and herbs to be used medicinally and nutritionally in medicines and various food preparations.

Conclusion

The daily use of supplements based on spices and herbs could supply essential antioxidants such as gallic acid, catechin and quercetin that could be highly helpful to counter the oxidative challenge imposed by free radicals which could eventually improve the quality of life by imparting general health and wellbeing.

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