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Screening of Some Selected Spices with Medicinal Value for Cu (II)-Ninhydrin Positive Compounds

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Abstract: Spices used in Indian traditional foods are rich in pharmaceutical and medicinal properties. Alpha-peptides wherein the carboxyl group is engaged in peptide bond formation are essential among the molecules present in the spices that provide medicinal properties. However, knowledge on the presence of such compounds in the common spices is limited. Cu (II)-ninhydrin reagent was used for the chromatographic identification of pharmaceutically important alpha-peptides in selected spices. Among the spices tested *Cuminum cyminum* (Cumin), *Coriandrum sativum* (Coriander), *Piper nigrum* (Black pepper), *Trigonella foenum-graecum* (Fenugreek), *Pimpinella anisum* (Aniseed), *Zingiber officinale* (Ginger), *Allium cepa* (Onion), *Elettaria cardamomum* (Cardamom), *Carum copticum* (Omum), *Brassica nigra* (Black mustard) and *Curcuma amada* (Mango ginger) were positive whereas *Allium sativum* (Garlic), *Papaver somniferum* (Poppy seed) and *Ferula asafoetida* (Asafoetida) were negative for Cu (II)- ninhydrin reagent. Among the spices that were positive for Cu (II)-ninhydrin, *Cuminum cyminum* (Cumin) appeared to possess higher levels of alpha-peptides in them. This reveals the fact that medicinally important alpha-peptides are prevalent in most of the common spices.

Key words: Spices, medicinal plants, cumin, alpha-peptides, Cu (II)-ninhydrin

INTRODUCTION

India is well known historically as a land of spices and continues to be one of the leading producers of spices and medicinal plants in the world (Prajapati *et al*., 2005). Flourishing trade existed for centuries on the sale of spices from the Indian sub-continent to the middle-east, Africa and Europe (Raychaudhuri, 1992). Increased demand for ethnic style foods has contributed to the rising usage of spices. The term aromatics includes all aromatic botanical substances used in food preparation including spices and condiments (Chomchalow and Henle, 1998). Most of the spices and herbs have great pharmaceutical value and they have been traditionally used in home based medicines (Shukla and Gardner, 2006). Medicinal plants are considered to be nature's gift and are the cheapest source of medicine for human and animal health. The medicinal flora is one of the richest cosmopolitan with highest therapeutic potentialities (Prajapati *et al*., 2005). Plants contain several alkaloids, steroids, glycosides, vitamins, hormones and antibiotics which may have marked pharmaceutical actions. Recent estimates state that about 25% of the drugs used presently come from the higher flowering plants (Farooqi and Sreeramu, 2001; Bhatia, 1997). Currently the global interest in natural plant products for use as medicine and health foods is increasing. This increase is mainly due to the disenhancement with the synthetic products of the modern chemistry and harmful side effects of many of the molecules used. In contrast, herbal products are perceived to be safe, environmentally friendly and free from side-effects (Kumar *et al*., 1997). Different plant parts such as rhizomes, bulbs, barks, leaves, buds, flowers, fruits and seeds are used for medicinal purposes (Pruthi, 1998).

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The majority of peptides occurring in nature come under the category of alpha-peptides, where in the alpha carboxyl group is engaged in the peptide bond formation. Alpha-peptides derived from medicinal plants have diverse biological functions in various fields (Ramachandramurty and Boopathy, 1994; Boschi *et al*., 1983; Monter *et al*., 1991; Robinson, 1992; Krebbers and Vandekerckhove, 1990). The thyrotrophic releasing hormone, for example is implicated in analgestic activity (Clapes *et al*., 1990). Application of short-chain peptides has been well advocated in clinical nutrition (Lisbeth, 1991). Certain alpha-peptides designed as growth factors have also been identified to have major role in the tissue regeneration process (Van Brunt, 1987). Spices and medicinal plants are one of the rich and unexploited sources of alpha-peptides (Ramachandramurty and Boopathy, 1994). The knowledge is limited on the presence of pharmaceutically important alpha-peptides in some of the important spices that are commonly used in ethnic foods. We have used Cu (II)-ninhydrin reagent for the identification of essential alpha-peptides in selected spices.

Unlike amino acids, small peptides such as Cu (II)-ninhydrin positive compounds are highly expensive and most of them are commercially not available in India. If the identification, separation and characterization methods for small peptides are standardized, these peptides can be easily isolated and supplied on demand for research as well as commercial purposes in India and abroad. Identification of the presence of Cu (II)-ninhydrin positive compounds has a great significance in commercialization of spices that have medicinal properties. In the present study, we have screened spices such as cumin, coriander, pepper, fenugreek, aniseed, ginger, garlic, onion, cardamom, poppy seed, omum, asafoetida, mustard and mango ginger for the presence of Cu (II)-ninhydrin positive compounds.

MATERIALS AND METHODS

Experiments were conducted at the department of biochemistry, PSG college of Arts and Science, Bharathiar University, India in 2002 to test the presence of Cu (II)-ninhydrin positive compounds in selected spices of medicinal value. Table 1 describes the spices used in the present study.

Preparation of Crude Extract

The spices were homogenized (1 g 15 mL^{-1}) thoroughly in a mortar and pestle for about 5 min with warm 80% aqueous ethanol (Ramachandramurty and Boopathy, 1994). The resultant ethanol extract was filtered through Whatmann No.1 filter paper. The filtered extract was then centrifuged at 3000 rpm for 10 min. The supernatant was then treated with 2% polyvinyl pyrrolidine (prepared by dissolving 2 g of polyvinyl pyrrolidine in 100 mL of distilled water), 1 mL for each gram of spice used as starting material and centrifuged at 3000 rpm for 10 min to remove the phenolic compounds. The clear supernatant obtained was used as the crude source for the extraction of the active principles.

Table 1: Spices used in the present study

Spices	Botanical name	Family
Cumin	*Cuminum cyminum*	Apiaceae
Coriander	*Coriandrum sativum*	Apiaceae
Black pepper	*Piper nigrum*	Piperaceae
Fenugreek	*Trigonella foenum-graecum*	Leguminosae
Aniseed	*Pimpinella anisum*	Umbelliferae
Ginger	*Zingiber officinale*	Zingiberaceae
Garlic	*Allium sativum*	Lilliaceae
Onion	*Allium cepa*	Lilliaceae
Cardamom	*Elettaria cardamomum*	Zingiberaceae
Poppy seed	*Papaver somniferum*	Papaveraceae
Omum	*Carum copticum*	Umbelliferae
Asafoetida	*Ferula asafoetida*	Umbelliferae
Mustard	*Brassica nigra*	Cruciferae
Mango-Ginger	*Curcuma amada*	Zingiberaceae

Development of Chromatographs

Circular paper chromatography was used for the identification of Cu (II)-ninhydrin positive compounds in the spices tested (Robinson and Targett, 1962; Ganguli, 1956). The crude spice extracts were spotted on a circular Whatmann No. 1 filter paper at the centre. Depending upon the number of samples, the paper can be demarcated. The diameter of the sample spotted was restricted to 0.5 cm by intermittent use of a hot air-dryer. The sample spotting may be done for 40 to 50 times to ensure sufficient concentration of the sample. The chromatography was carried out in an isopropanol: water (4:1 V/V) system by connecting a filter paper wick to the solvent system through a hole made at the centre of the circular paper. After the run, which takes about 30 to 45 min, the chromatogram was air-dried at ambient temperature (25 to 28°C) for 30 min. The air-dried chromatograms were uniformly sprayed with Cu (II)-ninhydrin reagent. This reagent was prepared by dissolving 300 mg of ninhydrin (1% W/V) and 217 mg of cupric nitrate in a mixture of 3 mL water and 1 mL glacial acetic acid and then the solution was made up to 30 mL with acetone. The sprayed chromatograms were then air-dried and heated at 65°C for 30 min.

Purification

Cu (II)-ninhydrin compounds were purified by ion-exchange chromatography using Dowex-50, a cation exchange resin. About 20 g of the Dowex-50 resin was suspended in 1 L of 0.5 N HCl (21.5 mL of concentrated HCl made up to 500 mL with water). Then, H^+ form of the resin was filtered and washed with distilled water until the filterate was free of acid and neutral in pH.

Setting the Column

The washed resin was then equilibrated to the pH level of 2.6 by suspending it in 100 mL of citrate-phosphate buffer of pH 2.6 for about one hour. This suspension was used to setup a column of about 2×3 cm.

Packing of the Column

For the purification of Cu (II)-ninhydrin positive compounds, 50 mL glass burette was used as a column. The column was packed by first making the bed, with glass wool and then attaching the tip of the burette to a rubber tube with pinch-cock arrangement. The column was fixed to stand in a vertical position. Dowex-50 resin suspended in citrate-phosphate buffer (pH 2.6) was poured into the column, taking care not to allow air bubbles and also to have an even distribution of the Dowex-50 resin and was allowed to settle maintaining the flow rate of 1.0 mL per 3 min. Once the column was set, it was thoroughly equilibrated with citrate-phosphate solution of pH 2.6.

Loading the Column

The crude ethanol extract was evaporated to dryness at room temperature. The dry extract was then dissolved in citrate-phosphate buffer at a pH level of 2.6. About 3 mL of the sample was loaded on the top of the column and the tap was opened to allow the sample to percolate through. The flow rate was then adjusted to 1 mL per 3 min.

Elution

The compounds of the extract that were bound to the column were eluted by increasing the pH of the column by using various buffers of different pH level. The eluant fractions were collected in 4 mL aliquots in serially numbered tubes. Seven such aliquots were collected in total. The elution buffers used were: citrate-phosphate buffer at a pH of 4 and 5; potassium-phosphate buffer at a pH of 7 and 8 and Tris-HCl buffer at a pH of 9, 10 and 11. These aliquots were spotted on a circular Whatmann. No.1 filter paper (7 cm) adjacently around a central hole and sprayed uniformly

with Cu (II)-ninhydrin reagent and heated to 65°C for 30 min to check the presence of Cu (II)-ninhydrin positive compounds in various aliquots based on the production of yellow chromophore with Cu (II)-ninhydrin reagent (Ramachandramurty and Boopathy, 1994; Robinson and Targett, 1962). The spice that showed highly positive for Cu (II)-ninhydrin reagent was then subjected to ion-exchange chromatography using Dowex-50, a cation exchange resin.

RESULTS AND DISCUSSION

The aliquots were tested for the presence of yellow chromophore with Cu (II)-ninhydrin reagent since this reagent produces yellow color with alpha-peptides. All amino acids and their carboxyl group derivatives like esters and amides including smaller peptides produce purple color with the classical ninhydrin reagent. This reagent was modified by adding cupric ion to the reagent, in order to distinguish qualitatively the carboxyl group derivatives from their amino acids by paper chromatography (Ganapathy *et al*., 1981). Table 2 describes the spices that were positive and negative for Cu (II)-ninhydrin reagent.

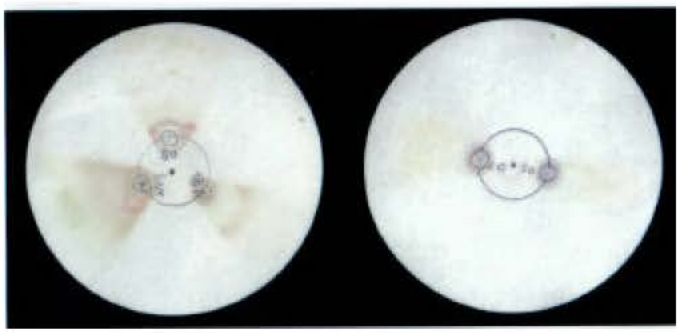
These results were evident from the appearance of yellow color with Cu (II)-ninhydrin reagent in circular chromatographs. The reaction between peptide and Cu (II)-ninhydrin is due to a complex formation rather than due to an oxidative reaction of the ninhydrin. Evidence for this phenomenon was provided earlier by Ganapathy *et al*. (1981) for a reaction sequence in which peptides react with Cu (II) ions to form a complex which then reacts with ninhydrin to give the yellow color chromophores.

Chromatograms of some of the important spices that revealed the presence of alpha-peptides were given in the Fig. 1. The circular chromatogram of *Cuminum cyminum*, *Coriandrum sativum* and *Piper nigrum* were exemplified in the Fig. 2(A) while the Fig. 2 (B) illustrated the chromatogram of *Zingiber officinale* and *Pimpinella anisum*. Among the spices that were positive for Cu (II)-ninhydrin reagent, *Cuminum cyminum* showed higher levels of alpha-peptides in them (Fig. 3).

The ethanol spice extract had amino compounds, amino acids and alpha-peptides together with glutathione. The Dowex-50 H^+ column was found to be effective in isolating alpha-peptides from other compounds by differential elution (Ramachandramurty and Boopathy, 1994). All the amino acids, amino compounds and glutathione appeared in the elute with lower pH values (4 to 7). While the alpha-peptides bound to the column appeared between pH values 9 and 11 (Ramachandramurty and Boopathy, 1994). Further hydrolysis experiments would give an idea of the nature of alpha-peptides. Fig. 3 reveals the elution of peptides at different buffers. Purification was done only from *Cuminum cyminum* (cumin) since higher amounts of yellow chromophore was observed in cumin during the initial screening.

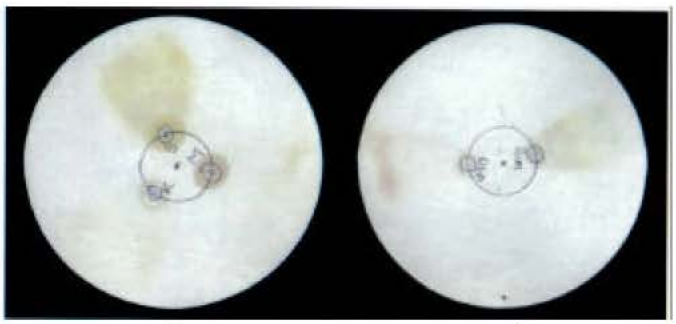
Table 2: Screening of Cu (II)-ninhydrin positive compounds in selected spices

Name of the spice	Cu (II)-ninhydrin positive/negative
Cuminum cyminum	Positive
Coriandrum sativum	Positive
Piper nigrum	Positive
Trigonella foenum-graecum	Positive
Pimpinella anisum	Positive
Zingiber officinale	Positive
Allium sativum	Negative
Allium cepa	Positive
Elettaria cardamomum	Positive
Papaver somniferum	Negative
Carum copticum	Positive
Ferula asfoetida	Negative
Brassica nigra	Positive
Curcuma amada	Positive



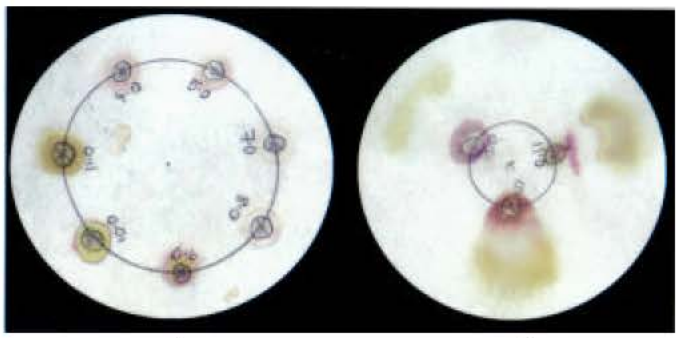
A B

Fig. 1: (A) Circular chromatogram of extracts of *Pimpinella anisum*, *Trigonella foenum-graecum* and *Zingiber officinale* sprayed with Cu (II)-ninhydrin reagent and (B) Circular chromatogram of *Allium cepa* varieties *cepa* (SO) and *aggregatum* (LO) extracts sprayed with Cu (II)-ninhydrin reagent



A B

Fig. 2: (A) Circular chromatogram of *Cuminum cyminum* (S), *Coriandrum sativum* (K) and *Piper nigrum* (M) extracts sprayed with Cu (II)-ninhydrin reagent and (B) circular chromatogram of *Zingiber officinale* (G) and *Pimpinella anisum* (S) extracts sprayed with Cu (II)-ninhydrin reagent

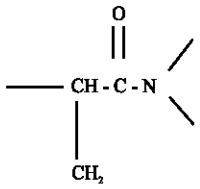


A B

Fig. 3: (A) Aliquots of *Cuminum cyminum* obtained by ion-exchange chromatography at different pH levels using Dowex-50 and (B) circular chromatogram of *Cuminum cyminum* in the aliquots at pH 10 and 11

From the Table 2 as well as the Fig. 1, 2 and 3, it is evident that among the 14 spices tested, 11 were positive and only 3 were negative for Cu (II)-ninhydrin reagent. This reveals the fact that most of the common spices are rich in medicinally important alpha-peptides. The presence of medicinal properties in different spices was also reported by several researchers (Arora and Kaur, 1999; De *et al*., 1999; Lai and Roy, 2004; Dankert *et al*., 1979; Elnima *et al*., 1983).

These results and also studies with several peptides having N- and C- terminal substitutions lead to the conclusion that the minimum structural requirement around the peptide linkage, for the formation of yellow chromophore with Cu (II)-ninhydrin reagent is as follows:



CONCLUSIONS

From the results obtained in the experiments, it was concluded that Cu (II)-ninhydrin positive compounds were identified in several spices that were commonly used in traditional Indian foods. However, spices such as *Allium sativum* (Garlic), *Papaver somniferum* (Poppy seed) and *Ferula asafoetida* (Asafoetida) were negative for Cu (II)- ninhydrin reagent. Alpha-peptides from *Cuminum cyminum* were partially purified by ion-exchange chromatography using Dowex-50, a cation exchange resin. The compounds purified in the present investigation could be small peptide, amino acid amine or amino acid ester whose structure has to be established after carrying out a systematic analysis. Further hydrolysis experiments should be carried out to know the amino acid composition. Moreover, sequence determination studies have to be done to completely standardize the protocols for isolation and purification of these compounds.

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