Vol. 1/2 (2001) 125 - 129

JOURNAL OF NATURAL REMEDIES

Evaluation of Tyrosinase inhibitory activity of some Indian spices

P. K. Mukherjee[1]*, S. Badami[1], A. M. Wahile[1], S. Rajan[2], B. Suresh[1]

1. JSS College of Pharmacy, Rocklands, Post Box 20, Ootacamund - 643001, Tamil Nadu, India
2. Medicinal Plant Collection Unit, Government Arts College, Ootacamund 643002, India

Received 22 April 2001; Revised and Accepted 18 June 2001

Abstract

Objective: Evaluation of tyrosinase inhibitory activity of spices. Materials and methods: Ten different spices considered as usual commodity in Indian food habits was screened for their inhibitory activity against tyrosinase. The mushroom tyrosinase inhibitory activity was determined by dopachrome method using L-DOPA as the substrate. Results: Amongst the spices tested four spices *viz.* Turmeric (*Curcuma longa*), cumin (*Cuminum cyminum*), black pepper (*Piper nigrum*), pipal (*Ficus religiosa*) showed the specific inhibitory activity against tryrosinase above 50% and out of them turmeric showed maximum inhibition which can be further explored for the characterization of the phytoconstituents. Other spices showed potential inhibition of tyrosinase activity. Conclusion: This finding could lead to the design and discovery of new tyrosinase inhibitors from Indian spices.

Key words: Tyrosinase inhibitor, alcohol extract, *Piper nigrum, Ficus religiosa, Myristica fragrance, Nigella sativa, Cinnamomum tamala, Murraya koenigii, Coriandrum sativum, Cuminum cyminum, Carum carvi, Curcuma longa.*

1. Introduction

Spices constitute a major portion of the Indian foods imparting flavor and preservative properties [1]. India is a veritable paradise for the large sources of spices through it different states due to the varied climatic zone and geography. Different spices have been reported for their varied therapeutic potential including analgesic, anti-inflammatory, anti oxidant, anti carcinogenic, anti microbial and to prevent microbial spoilage of foods [2-5]. The essential oils from spices like cumin, coriander, ginger etc has been found to possess antibacterial properties along with fungitoxic property [6-7]. Besides these capsiacin from *Capsicum anum* has been found to be a potential inhibitor of *Helicobacter pylori* - a potent gastric pathogen. Almost all the spices used in Indian food have been shown to possess astringent and preservative properties within them.

*Corresponding author
E-mail: mukherjeepk@rediffmail.com

Results from several studies on the physiological functions of the enzyme Tyrosinase (EC 1.14.18.1) have suggested a wide range of potential applications for its inhibitor [8].

Tyrosinase is one of the key enzymes involved in the molting process of insects, and investigating inhibitors of tyrosinase may provide important clues for developing new insect control agents. It also involves in unfavorable darkening of food products by enzymatic oxidation of phenolic compounds to corresponding o-quinones.

Therefore, its inhibitors may have potential uses as food additives. Tyrosinase are important for the pigmentation of the skin. In the senile cataract, de-maturation of the nuclear fibers gives amines. These amines undergoes oxidative polymerization leading to the melanin formation in presence of oxygenase enzyme and the deposition of the melanin leads to cloudiness.

Thus screening of tyrosinase inhibitors is important in the light of melanoma, senile cataract and development of insecticides and food preservatives [9, 10].

In man, potent tyrosinase inhibitors, such as Kojic acid derivatives have been used as whitening agent in cosmetic production, due to their ability to suppress dermal melanin production. Several classes of natural products have been found to possess tyrosinase inhibitory activity [11, 12].

Based on the varied uses of the spices and keeping the interest of the tyrosinase inhibitors in mind, the present study was undertaken to evaluate the tyrosinase inhibitory potential of the spices which are used most commonly in Indian food and thereby to evaluate their anti pigmentation potential through the enzymatic activity.

2. Materials and methods

2.1. Collection of Plant Material

Ten different spices were used in this study. The dried seeds of *Piper nigrum, Ficus religiosa, Myristica fragrance* and *Nigella sativa* respectively; dried leaves of *Cinnamomum tamala* (tejpatra), fresh leaves of *Murraya koenigii* (Curry leaf); dried fruits of *Coriandrum sativum* (coriander), *Cuminum cyminum* (cumin), *Carum carvi* (caraway); rhizome of *Curcuma longa* (turmeric) were procured from local market of Nilgiris district, Tamilnadu, India. They were identified by Medicinal plant Collection Unit, Govt. Arts College, Ootacamund India. A voucher specimen of each has been kept in our laboratory for future reference.

2.2. Preparation of Plant Extract

The respective plant parts of the individual spices were dried and pulverized separately by a mechanical grinder, passed through 40 mesh sieve and stored in a closed vessel for future use. The powdered plant materials of each plant were then extracted separately with methanol by maceration. These extracts were then concentrated and dried under reduced pressure. The semi-solid mass (solvent free) thus obtained was used for the experiment. The yield was 2.8%, 3.5% w/w for the leaves of *Cinamommum tamala* (tejpatra) and *Murraya koenigii* (Curry leaf) respectively; 3.2%, 2.4%, 4.2%, 3.8% w/w for seeds of *Piper nigrum* (black pepper), *Ficus religiosa* (pipal), *Myristica fragrance* (nutmeg), *Nigella sativa* (black cumin); 3.6%, 4.2%, 3.2% w/w for fruits of *Coriandrum sativum* (coriander), *Cuminum cyminum* (cumin), *Carum carvi* (caraway); 4.3 % w/w for the rhizome of *Curcuma longa* (turmeric) respectively.

The methanol extracts of all the spices on qualitative chemical tests and TLC characteristics showed the presence of essential

oils, steroids and flavonoids. The individual solvent extract was further diluted with dimethyl sulphoxide (DMSO) to the required concentration for the evaluation of their inhibitory activity against tyrosinase.

2.3. Chemicals and Instruments Used

Tyrosianse (EC 1.14.18.1) of 25000 units per mg was procured from Sigma Chemical Company, St Louis, USA as a lyophilized powder. 3,4-Dihydroxy-L-phenylalanine (L-DOPA) was obtained from Loba-Chemie, Mumbai; Methanol and DMSO and other chemicals were of AR grade and obtained from E. Merck, Mumbai, India. The Spectrophotometer used was of Perkin Elmer model 200.

2.4. Tyrosinase Assay

Mushroom tyrosinase activity was determined by the dopachrome method using L-DOPA as the substrate [11]. The reaction mixture (3ml) containing 0.5mM L-DOPA, 20mM phosphate buffer (pH 6.8), 100 units of mushroom tyrosinase and the sample in DMSO-water mixture at a concentration of 1 mg/ml was incubated at 25°C for 10 minute. A control reaction was carried out with out the test sample. The absorbance was measured at 475 nm before and after the incubation [13]. The percentage inhibition of tyrosinase was calculated using the formula:

Tyrosinase inhibition (%) = (A-B)/A x 100

Where,

A = the absorbance of the control before and after incubation; B = represents the difference in the absorbance of the test solution before and after incubation.

The same determination was performed for five times for all the extracts and the results were expressed as the Mean ± SEM from five determination.

3. Results

The tyrosinase inhibitory activity of alcoholic extracts of ten spices commonly used in the Indian food habits tested in a reaction mixture has been shown in Table 1.

The results (Table 1) indicate that all the spices used in this investigation except nutmeg showed potential activity on mushroom tyrosinase with varied degree. The methanol extracts of rhizomes of *Curcuma longa* (turmeric),

Table 1.
Tyrosinase inhibitory activity of some Indian spices

Name of the spices	Spices	Part used	Family	Inhibition(%) (Mean ± SEM) *
Balck cumin	*Nigella sativa*	Seeds	Ranunculaceae	55.0 ± 0.19
Black pepper	*Piper nigrum*	Seeds	Piperaceae	58.20 ± 0.16
Caraway	*Carum carvi*	Fruits	Umbelliferae	58.34 ± 0.34
Coriander	*Coriandrum sativum*	Fruits	Umbelliferae	47.76 ± 25 0
Cumin	*Cuminum cyminum*	Fruits	Umbelliferae	64.17 ± 0.30
Curry leaves	*Murraya koenigii*	Leaves	Rutaceae	30.24 ± 0.05
Nutmeg	*Myristica fragrance*	Seeds	Myristacaceae	00
Pipal	*Ficus religiosa*	Seeds	Moraceae	53.73 ± 0
Tejpatra	*Cinnamomum tamala*	Leaves	Lauraceae	25.0 ± 0.30
Turmeric	*Curcuma longa*	Rhizomes	Zingiberaceae	88.56 ± 0.19

*Each determination was an average of five determinations and was expressed as Mean ± SEM.

Cuminum Cyminum (cumin), *Carum carvi* (caraway), *Piper nigrum* (black pepper), *Nigella sativa* (black cumin), *Ficus religiosa* (pipal) showed more than 50% inhibition. Out of all the spices tested the rhizomes of *Curcuma longa* (turmeric) showed maximum efficacy of 88.56 % inhibition of tyrosinase activity.

4. Discussion

The colour of mammalian skin is determined by a number of factors, the most important of which is the degree and distribution of the melanin pigmentation. Melanin biosynthesis inhibitory compounds are useful not only as skin whitening agent to be used in cosmetics but also as a remedy for disturbances in pigmentation. Tyrosinase (Phenol oxidase) is known to be a key enzyme for melanin biosynthesis in plants, microorganisms and mammalian cells [14].

Tyrosinase inhibitory activity of different plant extracts has been reported [9,15] and tryrosinase inhibitors have been tested in cosmetics and pharmaceuticals as a way of preventing over production of melanin in epidermal layer. Tyrosinase catalyses the oxidation of L-tyrosine to L-DOPA and L-DOPA to dopaquinone [16]. These reactions are the initial steps of melanin biosynthetic pathways and have been the target for the inhibition of melanin biosynthesis in the course of searching for anti-hyper pigmenting agents. Spices are used extensively in the Indian food habits irrespective of the region or state they belong. The anti-microbial, preservative properties of different spices have been reported [5, 3].

This study proves the efficacy of these spices to be more useful in herbal cosmetics as it is mostly used in skin care preparation for its property of promoting skin health and to improve its complexion [17]. The tyrosinase is one of the most important key enzymes in the insect molting process, and investigation on its inhibitors may be important in finding alternative insect control agents.

Thus, this investigation led us to search for a naturally occurring tyrosinase inhibitors from Indian traditional medicine, which can further be exploited for its possibly responsible phytoconstituents.

5. Acknowledgements

The authors are thankful to The All India Council for Technical Education (AICTE), Govt. of India, New Delhi for financial support through AICTE Career Award for young Teacher [F. No. 1-52/CD/CA (8)/96-97] to Dr. Pulok K. Mukherjee. Thanks are due to his holiness Swamiji of JSS Maha-Vidyapeetha, Mysore, India for providing the necessary facilities of work.

References

1. Ayres JC, Mundt JO, Sandine WE. (1980) *Microbiology of Foods,* H. H. Freemen, San Francisco, USA; 339-410.
2. Curtis OF, Shetty K, Cassagnol G, Peleg M. (1996) *Food Biotechnol.* 10: 55-73.
3. Hosono A. (1996) *Wissenschoft Technol.* 29: 286-288.
4. Daw ZY. (1994) *Chemie Mikrobiol. Technol. Lebensmittel.* 16: 129-135.
5. De M, De AK, Banerjee AB. (1999) *Phytother. Res.* 13: 616-618.
6. Meena MR, Sethi VJ. (1994) *Food Sci. Technol.* 31: 68-71.

7. Tewei R. (1994) *Indian Perfumer* 38: 98-104.

8. Gelder VCWG, Flurkey WH, Wichers HJ. (1997) *Phytochemistry* 45: 1309-23.

9. Lida K, Hasa K, Shimomura K, Sudo S, Namba T. (1995) *Planta Med*. 61: 425-428.

10. Kubo I. (1997) In: Hedin PA, Hollingworth RM, Master EP, Miyomoto J, Thompson DG. (Eds.) *Phytochemicals for Pest Control*, American Chemical Society: Washington; 311-26.

11. Kubo I, Hori IK. (1999) *Planta Med.* 65: 19-22.

12. Seo B, Yan J, Lee S, Kim M, Hwang K, Kim J, Min KR, Kum YD. (1999) *Planta Med.* 65: 683-686.

13. Miranda M, Urbani G, Vito LD, Botti D. (1978) *Biochem. Biophysiol. Acta* 585: 398-401.

14. Shimizu K, Kondo R, Sakai K. (2000) *Planta Med.* 66: 11-15.

15. Likhitwitayawuid K, Sritularak B, Ernamkul WD. (2000) *Planta Med.* 66:275-277.

16. Hearing VJ. (1987) *Methods in Ezymology,* Academic Press: New York; 154-165.

17. Dey AC. (1980) *Indian Medicinal Plants Used in Ayurvedic Preparation,* Popular Prakashan: Mumbai; India; 336-410.