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SCREENING OF PLANT PARTS FOR ANTI-TYROSINASE ACTIVITY BY TYROSINASE ASSAY USING MUSHROOM TYROSINASE

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ABSTRACT

The aim of this work was to analyse the inhibitory activity of 30 plants using mushroom tyrosinase inhibition method. Plant extracts were prepared in hexane, ethyl acetate, methanol and water The results showed that two plant extracts, Citrus sinensis in ethyl acetate and Vitis vinifera in water, exerted a considerable level of in vitro mushroom tyrosinase inhibition (975.75% and 690.43% respectively) compared to positive controls of kojic acid in the same solvent systems (96% inhibition in Ethyl acetate and 10% in water). Further study is needed to identify phytochemical compounds with anti tyrosinase activity in Citrus sinensis in ethyl acetate and Vitis vinifera in water.

Keyword: PPO, L-DOPA, tyrosinase inhibition, plant screening, phytochemical compounds.

Tyrosinase is a multicopper monoxygenase enzyme with wide distribution in bacteria, fungi, higher plants and animals. It is also known as the polyphenoloxydase (PPO) (Sung-Yum Seo *et. al*, 2003). It's function include monophenolase (cresolase) and diphenolase (catecholase) activity (Kubo *et. al*, 2000). It is involved in biosynthesis of melanin and catalyses the o-hydroxylation of tyrosinase (monophenol) to 3,4-dihydroxyphenyl alanine or DOPA (o-diphenol). Oxidation of DOPA to dopaquinone (o-quinone) can then be transformed into the melanin pigments through a series of enzymatic and non-enzymatic reactions (Prota, 1992).

Tyrosinase is responsible for pigments of the skin, eyes and hair in mammals. In insects the enzyme is essential for the sclerotization of the exoskeleton, wound healing and parasite encapsulation (Kittisak, 2007). In plants, tyrosinase is localized in the chloroplasts of healthy plant tissues, whereas its substrates are contained in the vacuole. Brushing, peeling or crushing of the plant tissues leads to the loss of this compartment, allowing tyrosinase mediated browning reactions to take place. The enzymes are involved in the pigmentation and are important factors in wound healing and primary immune response (Harald *et. al*., 2005). Natural products containing melanin synthesis inhibitory activity are of interest with their potential cosmetic applications, for example skin-whitening or anti-browning preparations. Local hyperpigmentation can be found in various skin conditions including lentigo (a flat brownish pigmented spot on the skin), nevus (nodular lesion usually congenital), and ephelis (freckle). Inflammatory conditions, e.g. acne and eczema, can also cause hyperpigmentation. Another common hyperpigmentation is melasma, a localized facial hyperpigmentation, appearing during pregnancy or the course of hormone use.

Due to everyday growing market of cosmetic industry and increasing demand of better food processing techniques anti-tryosinase compounds are need of the hour. Various sources of anti-tryosinase compound have been reported but plants source needs to be safe and easily available. Several compounds known to possess anti-tyrosinase properties include arbutin, a naturally occurring beta-d-glucopyranoside of hydroquinone (Maeda *et. al*., 1996).

In the present study we compared tyrosinase inhibitory activity using mushroom tyrosinase inhibition method on 30 plants with specific parts purchased from a market in Meerut in India. Plant parts were extracted with 3 different organic solvents (ethyl acetate, hexane and methanol) and deionised water against a well known positive control, kojic acid. Quantitative analysis on anti-tyrosinase activity was carried out using UV spectrophotometry to

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quantify dopachrome, and intermediate product in the pathway of melanin synthesis (Narisa Kamkach, 2007).

MATERIAL AND METHOD

The plants with their specific parts (Table 1) were purchased from a market in Meerut in India. Their fresh and dry parts were used as per the requirement in the experiment. Plant materials were cleaned with water. They were sliced and then ground by Mortal pestle. Plant extracts were prepared in hexane, ethyl acetate, methanol and water. To prepare plant extracts, 25 g of fresh plant materials were extracted by means of maceration (cold extraction) at 30 °C for 12 h in a 100 mL solution of hexane, ethyl acetate and methanol. All plant extracts were then obtained after filtration. Finally test sample solutions were prepared for all plant extracts.

L-DOPA and Mushroom Tyrosinase were purchased from Sigma Chemical. 20 µL of mushroom tyrosinase (1000 U/mL), 20 µL of 0.1 M phosphate buffer (pH 6.8) and 100 µL of the test sample solution (20%) containing 20 µL of plant extracts, were mixed (called sample solution with enzyme). Sample solutions without enzyme were also prepared by repeating all previous steps but with no plant extracts added. Blank solutions with and without enzyme were also prepared with no test sample solution added. We also prepared positive controls of 0.5 mg/mL kojic acid solutions (with water), with and without enzyme. Twenty 20 µL of 0.85 mM L-DOPA solution as the substrate was added into every sample and blank. These assay mixtures were incubated at 25 °C for 10 min. The amount of dopachrome produced in the reaction mixture was measured at 475 nm (e475 = 3600 M−1cm−1) using the microplate reader (Zenyth 2000, Anthos Labtech Instrument). Percent inhibition of tyrosinase activity was calculated as the following:

$$\% \text{ tyrosinase inhibition} = \frac{(A - B) - (C - D) \times 100}{(A - B)}$$

Note:
A = absorbance of blank solution with enzyme
B = absorbance of blank solution without enzyme
C = absorbance of sample solution with enzyme
D = absorbance of sample solution without enzyme.

RESULT AND DISCUSSION

All four types of extracts were assayed with the help of spectrophotometer (Jasco V-530) the reading with and without extract were taken at 475 nm. With the help of above given formula percentage of inhibition was calculated. All the percentage inhibition values were given in the table 1 below. Inhibition of tyrosinase by kojic acid (positive control) varied by types of organic solvents, from 65% for kojic acid in methanol to 96% for kojic acid in ethyl acetate (Kaatz *et.al.* 1999, Cabanes *et.al.,*1994). Four of these 30 plants presented strong inhibition of tyrosinase with 50 - 90% inhibition comparable with those of kojic acid. When considering each type of solvent, four plant extracts in three different organic solvents and deionized water exhibited relatively high tyrosinase inhibition levels; 975.75% in *Citrus sinensis* (ethyl acetate), 690.43% in *Vitis vinifera* (deionised water), 509.16% for *Fragaria ananassa* (Ethyl acetate), 473.18% *Curcuma longa* (ethyl acetate), and 284.50% *Morus nigra* (Ethyl acetate). These inhibition levels were consistent with the solubility parameters of individual organic solvents (δ-SI); 29.7 for methanol, 18.2 for ethyl acetate and 14.9 for hexane. It has been known that substances found in plants with high anti-tyrosinase inhibition were mostly associated with α–arbutin. α–Arbutin, or 4-hydroxy phenyl α-D-glucopyranoside, is the anomer of the naturally occurring arbutin. The substance is a potent suppressor of melanin synthesis in human skin without apparent side effects. It has been reported that α-arbutin inhibits human tyrosinase much more effectively than does arbutin (Funayama *et. al*.,1995). To our knowledge, the previous phytochemical investigations of *Citrus sinensis* and *Vitis vinifera* did not reveal the presence of natural compounds described for their anti-tyrosinase activity. Therefore these two plants can be a potential source of further investigation for new anti-tyrosinase inhibitor activity including other plants also. The expected active constituents could be aromatic aldehyde, aromatic acid, or polyphenol since these compounds consist of

hydrophobic parts that could act as the competitive inhibitors in melanin synthesis. Further biological investigations on human melanocytes should be done to confirm these activities. Some other plants shown in the Table-1 showing significant result in mushroom tyrosinase inhibition are *Capsicum annuum* (capsicum), *Actinidia deliciosa* (Kiwi), *Musa acuminate balbisiana* (Banana), *Aloe vera* are also

Table 1: Inhibition percentages of different organic solvents and aqueous extracts.

Scientific name	Parts Used	Hexane	Ethyl acetate	Methanol	Water
Carica papaya	With peel	101.32%	127.07%	106.16%	49.16%
Carica papaya	Raw	*	79.90%	*	70.49%
Carica papaya	Seeds	*	112.17%	*	**204.57%**
Carica papaya	Without peel	*	**211.81%**	*	98.54%
Carica papaya	Ripe	*	187.50%	*	-181.50%
Raphanus sativus Linn.	Root	95.52%	70.63%	99.05%	48.43%
Physalis peruviana	Fruit	117.76%	143.72%	96.12%	174.75%
Citrus sinensis	With peel	102.63%	**975.75%**	116.50%	51.51%
Citrus sinensis	Without peel	*	100.38%	*	-86.69%
Citrus limon	Leaves	101.45%	59.08%	77.69%	118.00%
Cocos nucifera	Water	102.77%	88.30%	96.01%	102.78%
Vitis vinifera	Without peel	*	101.05%	*	**690.43%**
Capsicum annuum	Without seed	69.28%	**262.50%**	69.88%	107.14%
Actinidia deliciosa	Fruit	104.62%	104.27%	81.18%	**261.01%**
Glycyrrhiza glabra	Stem	124.68%	128.38%	85.28%	101.12%
Syzigiun cumunii	Leaves	*	136.00%	*	68.54%
Nelumbo nucifera	Stem	*	74.68%	*	97.05%
Vaccinium vitis idaea	Fruit	104.65%	85.52%	96.83%	88.20%
Fragaria ananassa	Fruit	92.78%	**509.16%**	88.39%	84.52%
Aloe vera	Leaves	*	**254.62%**	*	98.96%
Zingiber officinale	Root	43.52%	191.99%	18.58%	90.04%
Eriobotrya japonica	Fruit	*	33.63%	*	81.80%

Curcuma longa	Natural	*	**473.18%**	*	177.34%
Curcuma longa	Market	*	10.35%	*	51.63%
Musa acuminate	Pulp	*	112.75%	*	**277.72%**
Musa acuminate	Peel	*	164.92%	*	168.39%
Eucalyptus globus	Leaves	*	85.61%	*	57.15%
Daucus carota sativus	Root	94.61%	62.26%	104.73%	83.29%
Tamarindus indica	Bark	*	74.12%	*	66.99%
Tamarindus indica	Pulp	*	47.82%	*	54.73%
Tamarindus indica	Pulp of sweet tamarind	100.99%	102.24%	96.11%	62.65%
Morus nigra	Fruit	*	**284.50%**	*	114.13%
Morus nigra	Leaves	*	97.27%	*	93.72%
Malus pumila	Apple	*	84.59%	*	112.63%
Lycopersicon esculentu	Fruit	*	99.16%	*	115.21%
Agaricus bisporus	Fruit	104.58%	**258.48%**	95.53%	**227.46%**
Cucumis sativus	Fruit	100.14%	196.52%	101.89%	23.88%
Daucus carota sativus	Root	*	78.66%	*	108.54%
Kojic acid	-	87.16%	96.21%	65.24%	82.01%

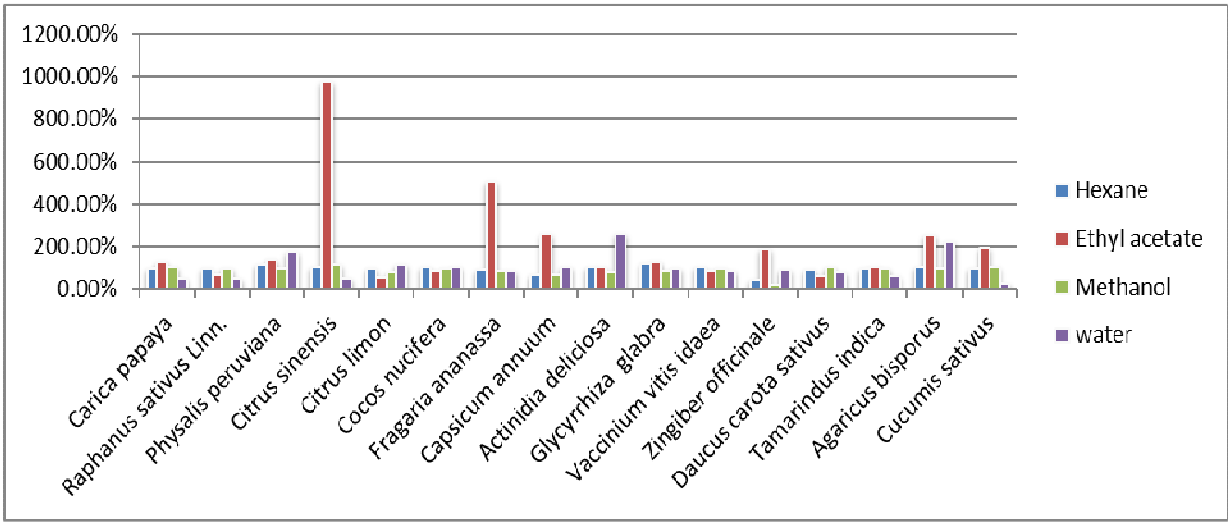
***Not Tested**

Table 2: Comparative study of some sample in four solvents

Scientific name	Parts	Hexane	Ethyl acetate	Methanol	Water
Carica papaya	With peel	101.32%	127.07%	106.16%	49.16%
Raphanus sativus Linn.	Root	95.52%	70.63%	99.05%	48.43%
Physalis peruviana	Fruit	117.76%	143.72%	96.12%	174.75%
Citrus sinensis	With peel	102.63%	**975.75%**	116.50%	51.51%
Citrus limon	Leaves	101.45%	59.08%	77.69%	118.00%
Cocos nucifera	Water	102.77%	88.30%	96.01%	102.78%
Fragaria ananassa	Fruit	92.78%	**509.16%**	88.39%	84.52%

Capsicum annuum	Without seed	69.28%	**262.50%**	69.88%	107.14%
Actinidia deliciosa	Fruit	104.62%	104.27%	81.18%	261.01%
Glycyrrhiza glabra	Stem	124.68%	128.38%	85.28%	101.12%
Vaccinium vitis idaea	Fruit	104.65%	85.52%	96.83%	88.20%
Zingiber officinale	Root	43.52%	191.99%	18.58%	90.04%
Daucus carota sativus	Root	94.61%	62.26%	104.73%	83.29%
Tamarindus indica	Sweet pulp	100.99%	102.24%	96.11%	62.65%
Agaricus bisporus	Fungus	104.58%	**258.48%**	95.53%	**227.46%**
Cucumis sativus	Fruit	100.14%	196.52%	101.89%	23.88%
Kojic acid	-	87.16%	96.21%	65.24%	82.01%

Graph 1: Tyrosinase inhibiting potential of various solvent extracts of certain plants



x- Sample

y- % inhibition of tyrosinase

Comparitive Graphical Study

Combined graphical study (Graph 1) of all four types of extracts provide much insight in our study. When all extracts were evaluated it was found that ethyl acetate extract especially of *Citrus sinenisis, Fragaria ananassa, Capsicum ammuum, Agaricus bisporus, Cucumis sativus* showing very significant inhibition compare with other. Contrary to other organic extracts aqueous extracts of *Actinidia deliciosa, Agaricus bisporus, Physalis peruviana* were better. As per the result obtained in our study we can conclude that different plant material express their anti tyrosinase activity differentially. Some were

better showing their effects in low polarity organic solvents like Ethyl acetate and methanol while rest were more inhibitory in hexane or aqueous solutions. solvents.

This could be attributed to presence of different active principals in different plants which shows variable solubility in different organic and aqueous

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