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BIO-REACTIVE PROPERTIES OF CITRUS WASTE: AN INVESTIGATION OF ANTIOXIDANT AND TYROSINASE INHIBITORY ACTIVITIES

MUSARRAT AKHTAR[1], LUBNA IQBAL[2*], MEHREEN LATEEF[2], BUSHRA NAWAB[1], MUHAMMAD SALEEM[2] AND NIGHAT AFZA[1]

[1] *Food & Marine Resources Research Centre, PCSIR Laboratories Complex, Karachi-75280*
[2] *Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi-75280*
[*]Corresponding authors e-mail: m_saleemqazi@yahoo.com

Abstract

The antioxidant potential as well as tyrosinase inhibitory activity of citrus fruit waste were determined. Fruit peels of 4 species of genus citrus viz. *Citrus sinensis* (Malta), *C. reticulate* (Orange), *C. paradisi* (Grape fruit) and *C. aurantifolia* (Lemon), were analyzed for these bio-reactive properties. Six different fractions of crude extracts of each citrus fruit peel were screened for antioxidant behavior through DPPH scavenging activity, while tyrosinase inhibition capacity was evaluated by using kojic acid as standard tyrosinase inhibitor. It was revealed that significant antioxidant potential was found in chloroform fraction of malta, orange and lemon, while chloroform and ethyl acetate, both fractions of grape fruit had 90% antioxidant activity at 1% concentration. Alcoholic fractions of orange, malta, and lemon showed 90, 87, and 69% tyrosinase inhibition respectively, while no tyrosinase inhibition was found in any fraction of grape fruit.

Introduction

Oxidative stress is a situation in which reactive oxygen species such as super oxide (O_2) hydrogen peroxide (H_2O_2), hydroxyl radical (HO) nitrogen oxide (NO), peroxynitrite (ONO_2) and hypochlorous acid (HOCl), are generated. These reactive oxygen species play an important role in degenerative or pathological processes, such as aging (Burns *et al.*, 2001), cancer, coronary heart disease, Alziemer's disease (Gay, 1990; Ames 1983; Diaz *et al.*, 1997); neurodegenerative disorder, atherosclerosis, cataracts and inflammation (Smith *et al.*, 1996). To protect cells and organs from the oxidative stress, induced by ROS, living organisms have efficient and highly sophisticated protective system, called antioxidant defensive system. It involves endogenous as well as exogenous components. These components function interactively and synergistically to neutralize free radicals (Percival *et al.*, 1998).

But sometimes this natural antioxidant mechanism can be inefficient and hence dietary intake of antioxidant compounds is important (Mushtaq *et al.*, 2005; Halliwel, 1994). There are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, it has been suggested that these synthetic compounds have some side effects (Terao *et al.*, 1994; Yildirm *et al.*, 2001). Therefore research on utilization of more effective natural antioxidants are desperately needed. The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products. Polyphenols are the most numerous group of antioxidant components, and they are present in fruits and vegetables (Fukushima *et al.*, 1983).

The aim of present study was to evaluate antioxidant potentials and tyrosinase inhibition activities of citrus fruit waste for which various parts of these plants have been used in folk lore so peels of these fruits were chosen to analyze. The main objective of this study is the determination of antioxidant activities of various extracts of citrus fruit waste by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) radical scavenging capability,along with the tyrosinase inhibition activities of various extracts of citrus fruits.

Materials and Methods

Plant material & preparation of extracts: Peels of citrus fruits were collected from local market of Karachi. The material taken, were shade dried and 1 kg of it was chopped into small parts with a blender. Then soaked in 10 L methanol at room temperature (three weeks x 3 times) and the filtrate was evaporated under reduced pressure at 45^{o}C to yield 112.5 g crude extract. For fractionation, 62.5g of the crude extract was dispersed in methanol (500 ml) and extracted with 500ml each of ethyl acetate, chloroform and butanol consecutively. All the fractions were evaporated under reduced pressure to obtain 190 mg ethyl acetate fraction, 165 mg chloroform fraction, 90 mg butanol fraction and used for enzyme inhibition and antioxidant activities.

Determination of DPPH radical scavenging activity: The free radical scavenging activity was measured by 1,1-diphenyl-2-picrylhydrazil (DPPH) (Soares *et al.,* 1997). The solution of DPPH of 0.3 mM was prepared in ethanol. Five microlitres of each sample of different concentration (1-2.5%) was mixed with 95 µL of DPPH solution in ethanol. The mixture was dispersed in 96 well plate and incubated at 37^{o}C for 30 minutes. The absorbance at 515 nm was measured by microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was determined in comparison with the methanol treated control.

$$\text{DPPH scavenging effect (\%)} = Ac - As/Ac \times 100$$

where Ac = Absorbance of control (DMSO treated), As = Absorbance of sample

Evaluation of tyrosinase inhibition activity: Tyrosinase inhibition assay was performed using kojic acid as standard inhibitor for tyrosinase in spectramax 340 microplate reader (Molecular Device USA) the compounds were screened for o-diphenolase inhibitory activity of tyrosinase using L-Dopa as substrate. 30 units mushroom tyrosinase (28 mM) was preincubated with compounds in 50 mM sodium phosphate buffer (pH 6.8) for 10 minutes at 25^{o}C. L-Dopa (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored for formation of DOPA chrome by measuring absorbance at 475 nm (at 37°C) for 10 minutes.

Results and Discussion

The objective of this investigation was to determine the antioxidant potential of peels of important fruits, commonly used and readily available in Pakistan. The assays performed pointed out the peels of fruits to be a rich source of natural antioxidants. Our study included peels of four fruits lemon, grape fruit, malta and orange, all four belong to citrus family, and revealed good potential to be used as antioxidant while ethanol fraction of malta and orange and ethyl acetate fraction of grape fruit showed remarkable tyrosinase inhibition activity which make them as strong candidate to be used in cosmetics.

Figure 1 showed that grape fruit crude extract as well as all five fractions reflected significant antioxidant potential i.e., ethanol fraction, chloroform and ethyl acetate showed 92, 91 and 90% respectively, followed by butanol fraction 60%, crude extract 59% and hexane fraction 40%.

Antioxidant capacity of Lemon, as shown in Fig. 2 revealed 70 % in crude extract while hexane 78%, butanol 80%, ethyl acetate and chloroform 91%, and ethanol fractions 92% respectively.

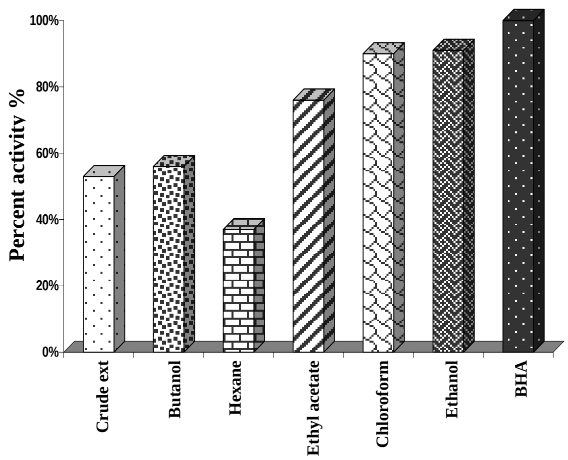

Fig. 1. Antioxidant activity of grape fruit extract and its fractions (1%).

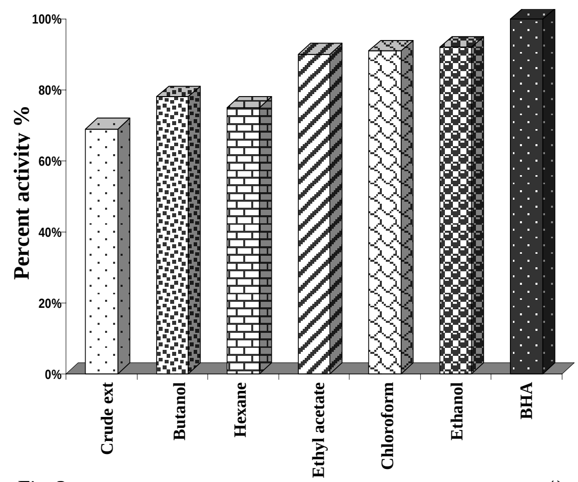

Fig. 2. antioxidant activity of lemon extract and its fractions (1%).

Antioxidant potential of Malta can be clearly seen from Fig. 3 that crude extract of Malta showed 60% of antioxidation, among the different fractions only chloroform showed 70% and ethanol 72% activity while others are devoid of antioxidant potential.

Figure 4 shows that antioxidant behavior of orange peels crude extract is not very promising; it showed 57% in crude extract while 70% in chloroform as well as in ethanol extract.

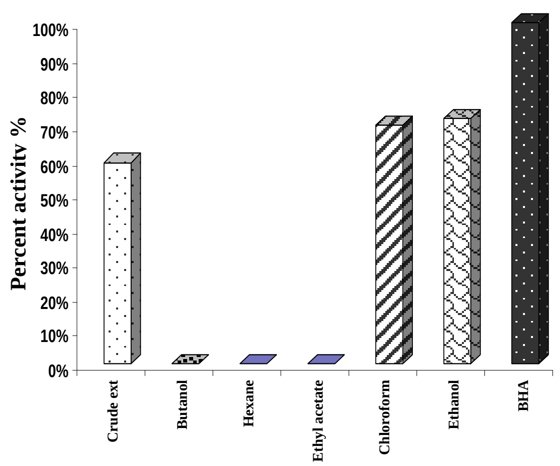

Fig. 3. Antioxidant activity of malta extract and its fractions (1%).

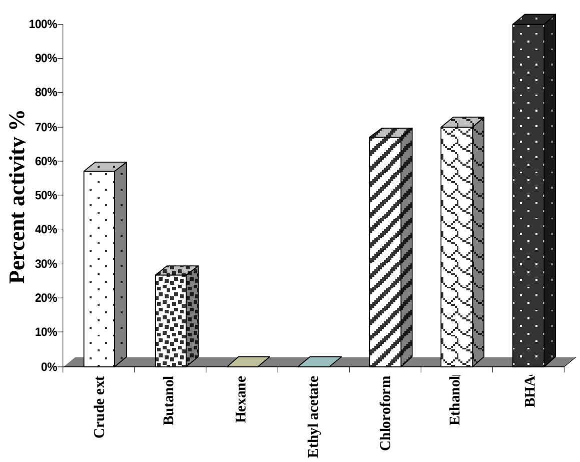

Fig. 4. Antioxidant activity of orange extract and its fractions (1%).

When all these extracts (four fruits) were screened for tyrosinase inhibition activities, it was noted that Lemon and Grape fruit did not show remarkable activity against tyrosinase, while ethanol fraction of Malta and Orange showed 92% and 91% inhibition respectively while only ethyl acetate fraction of Grape fruit showed 87% inhibition, followed by 85% by lemon extract with no activity in other fractions (Fig. 5).

The growing demand for natural antioxidants in food and cosmetic industries forces the search for new sources of these compounds. Numerous scientific investigations point at consecutive rich sources of antioxidants, especially among fruits, but only few of them involve waste parts of fruits (Boyer & Liu, 2004).

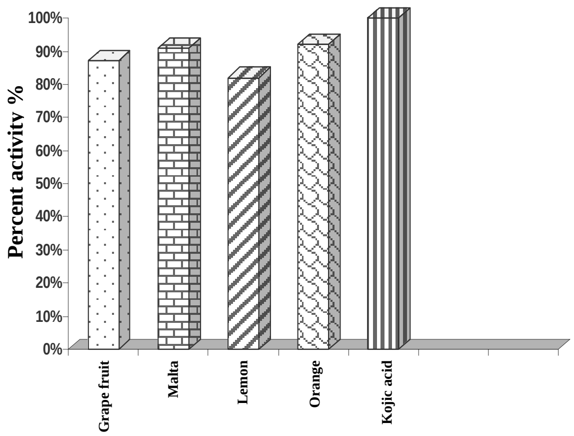

Fig. 5. Tyrosinase inhibition activity of citrus extract and its fractions (1%).

Fruits and vegetable wastes and by products, which are formed in great amount during industrial processing , correspond to a serious problem, as they exert an influence on environment and need to be managed or utilized. On the other hand they are very rich in bioactive components, which are considered to have a beneficial effect on health. For the last decades efforts have been made to improve methods and ways of re-using fruits and vegetables wastes (Duda-Chodak & Tarko, 2007; Jayaprakasha *et al.,* 2003).

The peel amount nearly half of entire citrus crop and is currently sold as cattle feed at marginal or no profit. This waste contains sugars, edible fibers and many other components that can be isolated or converted into value added products. Components from citrus peel reported to have biological activity (antioxidant, anticancer, cardio protective) (Anon., 2002). The antioxidant supplementation is a generally accepted method of prolonging the stability and storage life of foods. However the artificial/synthetic antioxidant compounds like BHA and BHT, have a limited allowance for food due to their potential carcinogenicity (Soares *et al*., 1997).

Recently, a global market demand has developed for skin whitening agents in many countries. Several tyrosinase inhibitors have been used in cosmetic industry as skin whitening agents. Unfortunately, many of these agents have not been proved effective. Thus many efforts are still needed to include a real tyrosinase inhibitor with significant activity.

Malta and Orange peels are rich source of tyrosinase inhibitor and regularly used as skin whitener in locally formulated “ubtans”. Our study also confirmed this by showing above 90% inhibition of tyrosinase activity by ethanolic fraction of Orange and Malta peels and 87% activity in ethyl acetate fraction of Lemon peels and grape fruit.

Ethanolic fractions of Malta and Orange showed significant antioxidant potential as well as strong tyrosinase inhibition activity. Both these properties make them strong and feasible candidate of any skin freshening and whitening formulation. The ultimate goal of such studies is to develop a high value functional food or neutraceutical product from citrus waste peels.

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