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Browning of Apple Can Prevent Growth of Candida Species

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ABSTRACT

Polyphenol oxidase(PPO) is a food enzyme which holds a very important place in the food industry mainly because of its impairing effects. This enzyme leads to the darkening of tissue when fruits are cut or any damage occurs which leads to the depreciation of nutritional value and food acceptability. It is not clear which roles this compound may have in cell metabolism. In plants, PPO contributes to defence mechanisms against pests and pathogens by producing toxic quinones that deter herbivory and inhibit microbial growth. In this report, the possible antifungal effects were examined. Analyses are carried out by properties of standard polyphenolic substances oxidized by PPO were assayed on Red delicious apple extract whose phenolics were previously isolated.

Keywords: Polyphenol oxidase, antifungal activity, Red delicious apple, minimum inhibitory concentration, *Candida albicans*, *Candida parapsilosis*

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INTRODUCTION

Polyphenol oxidase catalyzes the oxidation of phenolic compounds to quinones (Figure 1), which leads to the formation of brown pigments[1]. This activity is a key factor in the browning of fruits and vegetables when they are damaged or cut. PPO binds to phenolic compounds such as catechol tyrosine [2-4] that is present in the plant tissues. The enzyme then catalyzes the oxidation of these phenolic compounds into quinones where oxygen is used as co-substrate. Thereafter quinones polymerize spontaneously to form melanins, which results in the formation of browning of the tissue. There are certain factors, which affect PPO activity, such as pH and temperature, substrate concentration and most importantly inhibitors [4]. The optimum activity of PPO is observed at slightly acidic to neutral pH and varying temperature ranges depending on the source of the enzyme. A higher concentration of phenolic substrates can enhance PPO activity until a saturation point. Compounds such as ascorbic acid, citric acid, and sulfites can inhibit PPO activity hence reducing the browning of tissue.

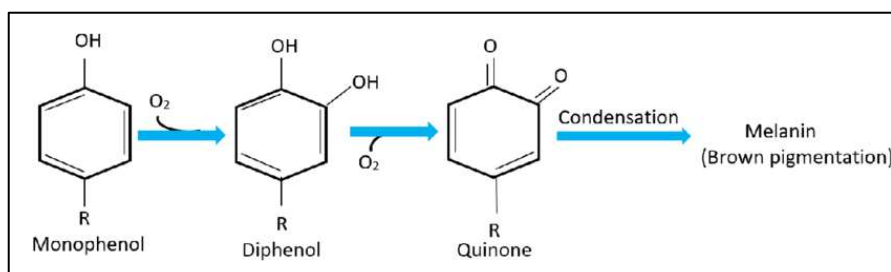


Figure 1: Oxidation of phenolic compounds

In this study, the possible antifungal action of ethanolic extract of *Malus domestica*(apple) was tested against American Type Culture Collection (ATCC) and Multidrug-resistant (MDR) strains of pathogenic fungi, *Candida* species[5-6]. Two ATCC *Candida* species were taken; *Candida albicans* (ATCC-14053) and *Candida parapsilosis*(ATCC-22019).

C. albicans is a type of fungus that is found in small amounts in our skin, mouth and intestines. Microbiome control the balance of candida. When candida is off-balance the yeast overgrows and causes infection. *C. albicans* is very sensitive to multiple environments that it encounters in the human host and forms hyphae in response to factors such as 37°C temperature, serum, CO_2 and O_2 tension, and neutral pH. Certain diseases are caused by *C.albicans*, which include oral thrush(white patches in the mouth and throat)[7], vaginal candidiasis (itching, irritation and discharge in the vagina), candidemia (bloodstream infection which leads to systemic candidiasis which affects organs like heart brain and eyes)[8], invasive candidiasis (causes severe infection in organs such as spleen, liver, kidney which is typically seen in immune compromised patients)[8-

9]. Mortality rates associated with these infections have not improved significantly and remain in the range of 30-40%. Systemic candidiasis causes more case fatalities than any other systemic mycosis[10].

C.parapsilosis [11] is a type of fungus that is found in our skin and gastrointestinal tract. Most of the time this strain of candida does not harm us but surgery, injury or disease can let it grow in places where it does not belong. Infections in your blood or internal organs with *C.parapsilosis* are called invasive candidiasis[12].*C.parapsilosis* can causes endocarditis (inflammation of the lining of the heart valve and chambers), peritonitis(inflammation of the lining of the stomach)[13], onychomycosis(nail infection). Rarely it can cause meningitis, arthritis, or UTI(urinary tract infection)[14]. Invasive fungal infections[15] result in substantial morbidity and mortality (0.4 deaths per 100,000 population). Hence, these diseases have a major impact on public health. Over the past decade, the prevalence of *C.parapsilosis* has dramatically increased. Reports indicate that *C. parapsilosis* is frequently the second most commonly isolated *Candida* species from blood cultures.

MATERIALS AND METHOD

Fresh Red apple was sliced and kept for 3 days for browning [Figure 6 (A)] in the presence of air. After that the brownish part which was seen due to the activity of PPO was collected by scrapping. The scrapped brownish remain was weighed to 1gm. Then scrapped material was crushed in mortar and pestle to prepare a paste. The crushed sample was transferred to a falcon tube and 10 ml ethanol was added [Figure 6(B)]. Vortexing was performed for 5 minutes and kept at room temperate for 48 hours, which was followed by centrifugation and supernatant was collected. Another extract of the brownish part from the apple was taken after leaving it for an hour[Figure 6(C)]. A similar procedure of adding ethanol to the extract and vortexing was done and kept at room temperature for 48 hours. Subsequently, centrifugation was done and the supernatant was collected. The final concentration of both samples was estimated to be 100 mg/ml. The UV (Ultraviolet) visible spectra (Figure. 2-5) reading was taken for both the ethanolic samples.

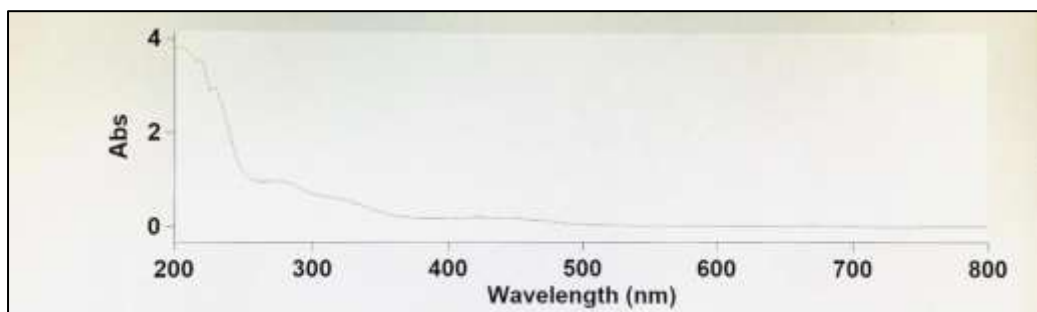


Figure 2: UV spectra reading of ethanolic sample of apple left for long browning

Sample Name:	MD
Collection Time	30-05-2024 00:18:00
Peak Table	
Peak Style	Peaks
Peak Threshold	0.0100
Range	300.0nm to 200.0nm
Wavelength (nm)	Abs
670.0	0.026
420.0	0.264
275.0	0.971
250.0	2.367
220.0	3.546
205.0	3.823

Figure 3: Peaks obtained from the spectra reading (Figure 2)

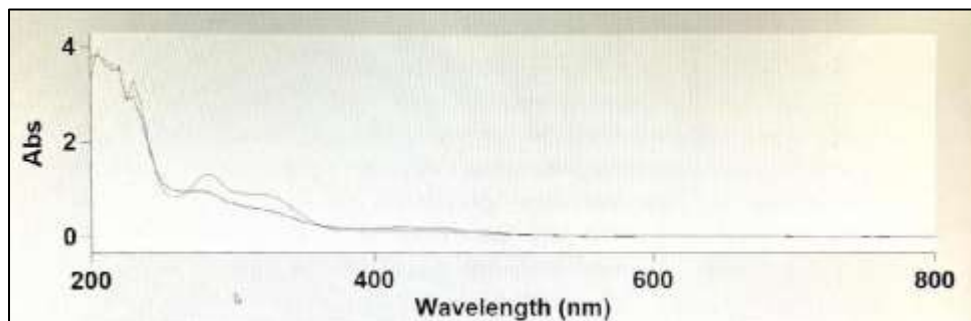


Figure 4: UV spectra reading of ethanolic samples of apple left for long browning (black line) and short browning (red line)

Sample Name:	MD 1HR
Collection Time	31-05-2024 00:20:33
Peak Table	
Peak Style	Peaks
Peak Threshold	0.0100
Range	300.0nm to 200.0nm
Wavelength (nm)	Abs
672.0	0.028
420.0	1.331
250.0	3.100
215.0	3.653
205.0	3.844

Figure 5: Peaks obtained from the spectra reading (Figure 4)



A

B

C

Figure 6: (A) Browning of apple (B) Ethanolic extract of apple left for short browning (C) Ethanolic extract of apple left for long browning

Determination of MIC values against the American Type Culture Collection (ATCC) and Multidrug resistant (MDR) strains of pathogenic fungi, *Candida* species.

The fungal isolates were made up of 0.5 McFarland opacity bacterial suspensions using Normal saline(NS) with VITEK® DENSICHEK®. The use of NS was to maintain the tonicity of the medium, which also prevents the lysis of the fungal cells. Firstly, 100 µl Mueller Hinton broth was added to each microwell of a sterile microtitre plate. Then 100µl of extract(long browning extract of the apple) was added to the first well of the first and third row and then serial dilution was done along the horizontal row up to the eighth well. The excess fluid was discarded from the eighth well. Therefore, in each step, there was a double dilution of the extract till the eighth well. After this 100µl of extract(short browning extract of the apple) was added to the first well of the fifth and seventh row and then serial dilution was done along the horizontal row up to the eighth well. Subsequently, the fungal suspensions in a fixed quantity(10 µl) were added to all eight wells. In the second, fourth, sixth and eighth rows similar dilutions were made with vehicle alcohol working as control and the fungal suspensions were also added.

C.albicans was added to the first, second, fifth and sixth rows, in all the wells individually. *C.parapsilosis* was added to the third, fourth, seventh and eighth rows in all the wells individually. Hence, in this way, in different rows, different fungal suspensions were added. The optical density of these wells was measured at 620nm at zero hours for the zero hour reading and then left for incubation overnight at 37° Incubator. The next day the 24-hour reading was measured. Change in optical density value was calculated by subtracting the 24-hour reading from the 0-hour reading. Then these values were compared with the control for determination of MIC values.

RESULTS AND DISCUSSION

The results of the study of MIC values show that in short browning (Figure 9-10) there was no antifungal activity, but in long browning (Figure 7-8) there was definite antifungal activity. Polyphenol oxidase which is a bioactive chemical is only induced when it is exposed long time. The MIC (minimum inhibitory concentration) value is less in long browning than in short browning.

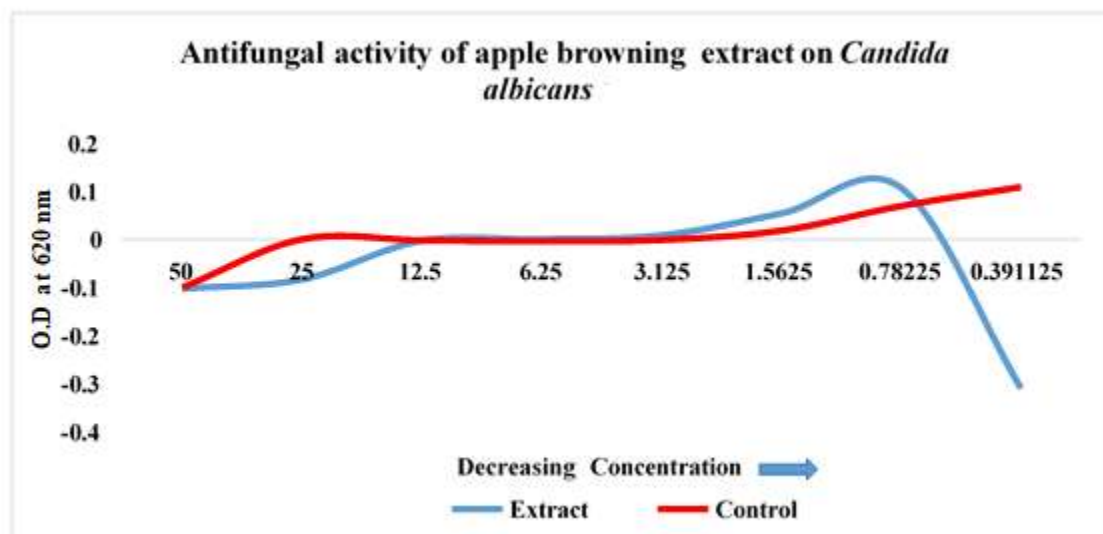


Figure 7: Showing effect of polyphenol oxidase from the apple extract (long browning) on the growth of *C. albicans* (MIC value is 0.39mg/ml) 1-50mg/ml, 2-25mg/ml, 3- 12.5mg/ml, 4- 6.125mg/ml, 5- 3.125mg/ml, 6-1.5625mg/ml, 7- 0.78125mg/ml, 8- 0.390625mg/ml

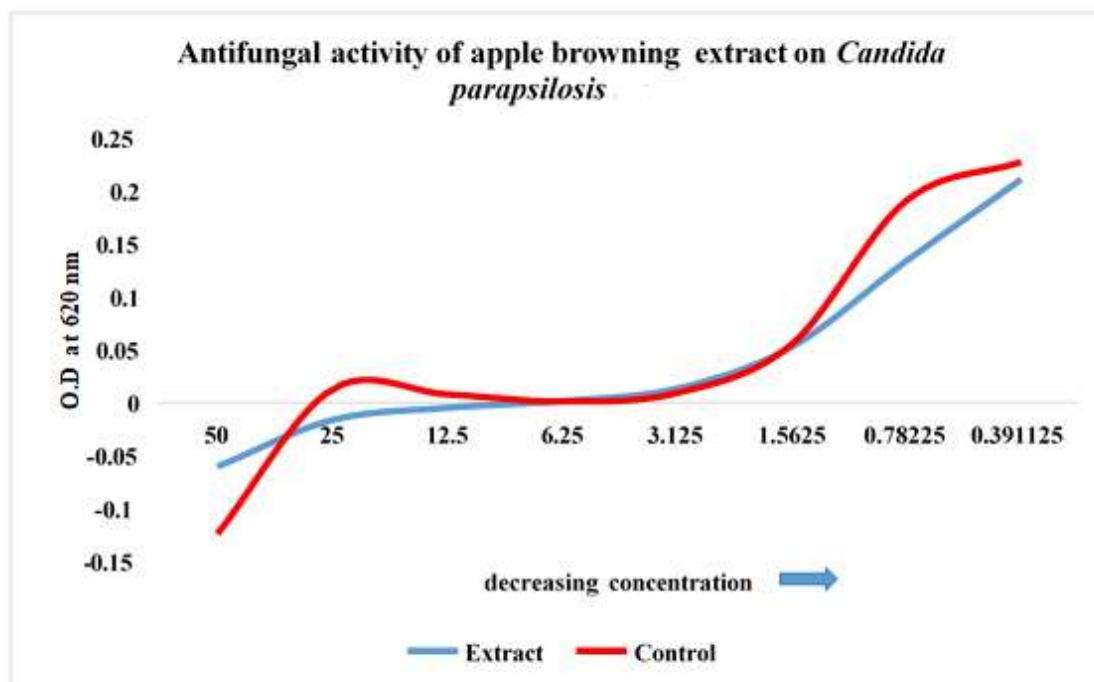


Figure 8: Showing effect of polyphenol oxidase from the apple extract (long browning) on the growth of *C. parapsilosis* (MIC value is 0.39mg/ml) 1-50mg/ml, 2-25mg/ml, 3- 12.5mg/ml, 4- 6.125mg/ml, 5- 3.125mg/ml, 6-1.5625mg/ml, 7- 0.78125mg/ml, 8- 0.390625mg/ml

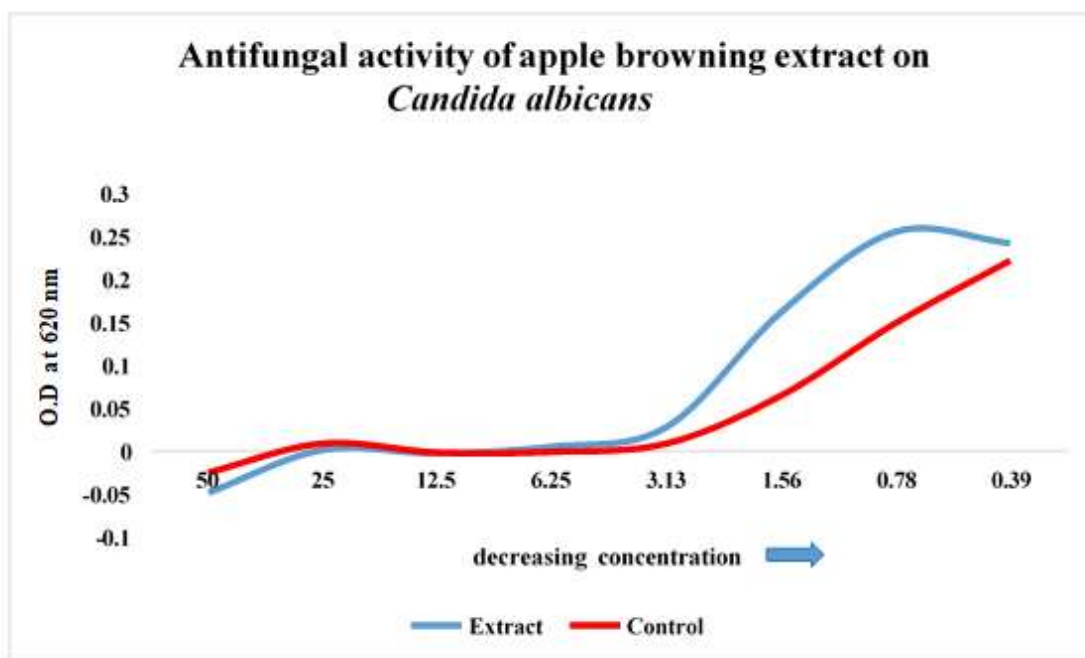


Figure 9: Showing effect of polyphenol oxidase from the apple extract(short browning) on the growth of *C.albicans* (MIC value is 50mg/ml)1-50mg/ml, 2-25mg/ml, 3- 12.5mg/ml, 4- 6.125mg/ml, 5- 3.125mg/ml, 6-1.5625mg/ml, 7- 0.78125mg/ml, 8- 0.390625mg/ml

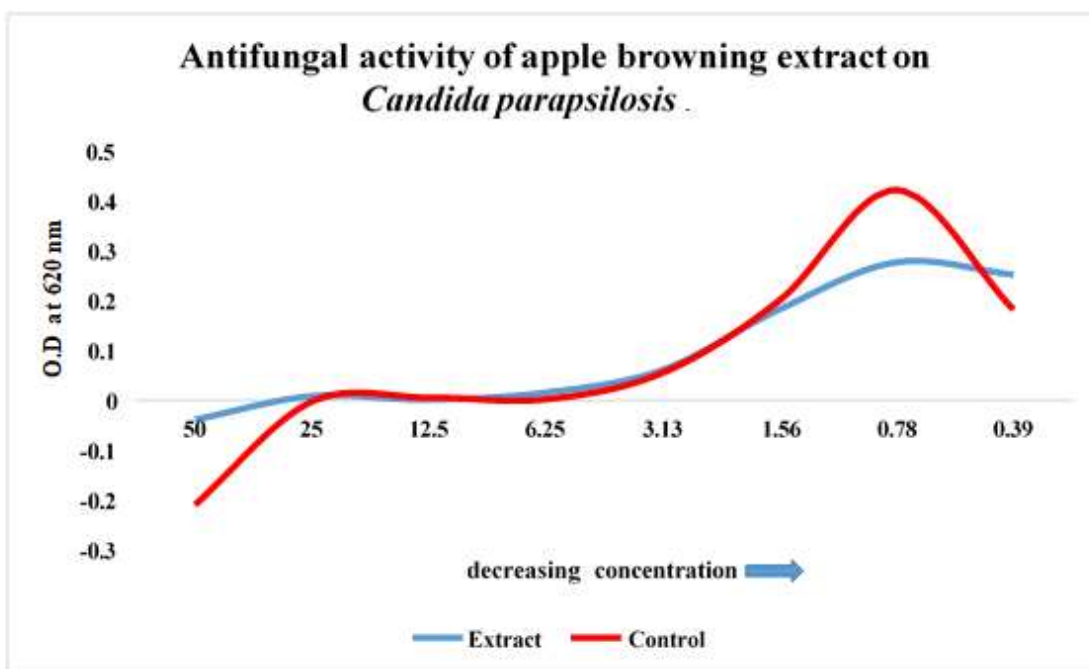


Figure 10: Showing effect of polyphenol oxidase from the apple extract (short browning)on the growth of *C.parapsilosis* (MIC >50mg/ml)1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4- 6.125mg/ml, 5- 3.125mg/ml, 6-1.5625mg/ml, 7- 0.78125mg/ml, 8- 0.390625mg/ml

From this study, we found that polyphenol oxidase production from the extracted sample of apples significantly inhibits the growth of candida species. We know there are many diseases which are caused by the candida species and some of them are fatal also. This finding sheds light on the inhibition of these infections caused by these species and can be useful as an antifungal element. This study also shows that long browning(Figure 7-8) has much more effect on the secretion of polyphenol oxidase than short browning(Figure 9-10). Hence long browning leads to more polyphenol oxidase production from the apple extracts which oxidises the phenolic compounds. Since a lower MIC value means less drug is required to inhibit the growth of fungal agents, drugs with lower MIC values are more effective antifungal agents. Hence the use of polyphenol oxidase can be an effective antifungal agent.

CONCLUSION

Polyphenol oxidase(PPO) of fruits leads to the darkening of tissue when fruits are cut or any damage occurs. In plants, PPO contributes to defense mechanisms against pests and pathogens. In this report, the possible antifungal effects of PPO was explored.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

Dr.Satadal Das designed the study procedure, analysed the data and corrected the manuscript. Ms. Ahana Talukdar performed the experiment under the guidance of Mr.Arup Kumar Dawn. Dr.Bhaskar Narayan Chaudhari and Dr.Partha Guchait analysed the data and corrected the manuscript.

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