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Gene Expression Analysis of Anti-oxidative Enzymes in Yeast against Oxidative Stress in Presence of *Cinnamomum zeylanicum.*

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ABSTRACT

Increased oxidative stress and impaired antioxidant defence mechanisms are believed to be important factors contributing to the cell damage and various diseases. The yeast *Saccharomyces cerevisiae*, is model organism to study various adaptive stress response mechanisms. In this study, we have reported the total phenol and flavonoid content of aqueous cinnamon extract are 481 mg GAE/100g and 300mg of RE/100gm, respectively. Total antioxidant capacity of aqueous cinnamon extracts by DPPH RSA, ABTS RSA and FRAP Assays were found to be 1058.75 mg and 5341.704 mg, 451.47 mg of TE /100 g, respectively. The spot assay revealed that the aqueous cinnamon extract protects the yeast cells from oxidative stress. It showed protection against ROS at both concentrations (0.2 mg/ml and 0.4 mg/ml). The expression of antioxidant enzymes, *SOD1*, *CTT1*, *CTA1* and *GSH1* were upregulated in stressed cells (1.3 to 2 fold) but yeast cells pre-treated with aqueous cinnamon extract showed a significantly decrease ($P \leq 0.05$) (1.1 fold) the expression at both concentrations (0.2 mg/ml and 0.4 mg/ml). The aqueous cinnamon extract showed protection against the oxidative stress in yeast model.

Key words: oxidative stress, antioxidant activity, cinnamon extract, yeast, gene expression

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INTRODUCTION

Reactive oxygen species (ROS) such as $O_2^{\bullet}$ And $NO_2^{\bullet}$ are by products of many oxygen dependent redox reactions. Higher levels of Free radicals (ROS) can result in deleterious consequences for the cell including lipid peroxidation, protein carbonylation and DNA damage [1]. These Damage to various biomolecules may be a risk factor for aging and various diseases like diabetes, CVD, cancer etc. Hydrogen peroxide (H_2O_2) is the most abundant reactive oxygen species in vivo, being continuously produced intracellularly as a by-product of the aerobic metabolism, or extracellularly by stimulated host phagocyte [2]. In multicellular organism, apoptotic cell death is an active and defined process. The impairment of apoptosis cause physiological and pathological cell death and increase the risk of cancer development. The budding yeast *Saccharomyces cerevisiae* are continuously exposed to reactive oxidants and oxidative stress occurs when the oxidants increases beyond the antioxidant buffering capacity of cells [3]. There are several methods established as marker of oxidative stress in cells caused either by excessive production of ROS and reduced antioxidant defence. The cellular oxidative stress response included the regulation of antioxidant enzymes gene expression, leading to increased antioxidant enzymes activities and, therefore, to a faster removal of the oxidants by the cell, protecting the cell against oxidative stress [4-7]. The cells have some defensive substance including superoxide dismutase (SOD), catalase (CAT), several peroxidases and antioxidants, such as ascorbate, tocopherol, uric acid, b-carotene and glutathione (GSH) against these ROS [8]. Other then cellular enzyme, dietary antioxidant molecules also act as "free radical scavengers" by preventing and repairing damage caused by ROS. In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals and antioxidants. Dietary antioxidants protect against oxidative damage to DNA, proteins, and lipids and have a significant impact on the regulation of gene expression [9].

Cinnamon (*cinnamomum zeylanicum*), a seasoning spice, is also a traditional medicine used to treat dyspepsia, gastritis, blood circulation issues and inflammatory disease. Cinnamon has many pharmacological properties, such as anti-inflammatory, antimicrobial, antidiabetic and antitumor activity [10-12]. Mancini-Filho et al. reported various extracts of cinnamon, such as ether, aqueous, and methanolic extracts that have shown considerable antioxidant activities [13] Volatile oils from *C. zeylanicum* showed significant biological activities [14]. Lin et al. reported the *in vivo* antioxidant activity of two different extracts, the ethanolic and hot water extracts of the dry bark of *C. cassia.* [15]. A various studies show that cinnamon plant has similar activity of insulin [16-17] and cholesterol and lipid lowering effect [18-19].

The role of the antioxidative response in adaption of yeast cell and altered their gene expression, we analysed aqueous cinnamon extract for their antioxidant potential in yeast in vivo.

MATERIALS AND METHODS

Chemicals and Reagents

ABTS (A-1888), DPPH (D-9132), Rutin (R-5143), Gallic acid (G-7384) and TPTZ (T-1253) Trizol (9424) were purchased from Sigma Aldrich-Germany and Trolox 56510 was purchased from Fluka. YEPD Broth and YEPD agar was purchased from Himedia, India. All RT-PCR kit purchased from Roche, USA. All other chemicals used were of analytical grade.

Preparation of extracts

The barks of *cinnamomum zeylanicum* was purchased from local market of Anand, Gujarat, India. It was then ground to a fine powder. Twenty grams of the powdered sample was then Extracted for 24 h in hot deionised water under continuous stirring at room temperature and then filtered through whatman no.1 filter. Cinnamon extract was stored at -20° C for further analysis.

Total Phenol Estimation

Folin Ciocalteu method was used to determine the total phenolic content of the Aqueous Cinnamon extracts [20]. Different aliquots of known concentration of gallic acid were taken as standard.

Flavonoid

Different aliquots of concentrated sample were used for estimation of flavonoid content [21]. Different aliquots of Rutin were treated as standard.

Ferric Reducing Antioxidant Power (FRAP)

Total antioxidant capacity of the Cinnamon extracts using FRAP assay was determined by the method of Benzie and Strain (1999)[22]. Different aliquots of Trolox were treated as standard and results were expressed in terms of TEAC (mg of Trolox Equivalent/100 g).

DPPH Radical Scavenging Ability

The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical, was determined by the method described by Brand-Williams et al, (1995)[23].The percent inhibition and IC 50 was calculated and Results were expressed in terms of TEAC (mg of Trolox Equivalent/100 g).

ABTS Radical Scavenging Ability

The radical scavenging ability of the extracts was determined using the modified ABTS radical decolorization assay [24]. The percent inhibition was calculated and results were expressed in terms of TEAC (mg Trolox Equivalent/100 g).

Antioxidant analysis in yeast model system

Yeast strains and growth condition

The yeast strain used in this study was *saccharomyces cerevisiae* MTCC 2636 purchased from IMTECH, Chandigarh, India. Yeast were routinely grown on YEPD medium [10 g/L yeast extract; 20g/L peptic digest of animal tissue; 20g/L dextrose, pH 6.5± 0.2] or YPD agar medium (15 g/L agar).

Yeast cell culture and stress induction

The yeast adaptive response assay was conducted with some modifications. The over grown culture of yeast cells were re-inoculated in three flasks of fresh 60 ml of YPD medium at 1% inoculum, containing or not aqueous extract of cinnamon (final concentration 0.2 mg/ml and 0.4mg/ml). Incubation was performed at 30˚C at 150 rpm (A600=0.6) to adapt the cells to the new medium, thus allowing the antioxidants to operate its protecting activity. After then 25 ml of yeast culture was divided and palate down at 8000 rpm for 5 min. Yeast cells were washed with 1X PBS (pH= 7.4). The oxidative stress induced by hydrogen peroxide (10mM) in this experiment as mentioned in group: (i) Control (ii) H_2O_2 (iii) CE 0.2 (iv) CE0.2 +H_2O_2 (v) CE0.4 (vi) CE0.4 +H_2O_2. All groups then incubated at 30˚C for 1 hour at 150 rpm. Cells were then washed with 1X PBS and quickly collected for the RNA extraction. Cell viability was analysed by spotting 5µl of yeast cells from each group on YEPD agar plate. To choose the doses of the cinnamon extract used in the treatment, cells were exposed to H_2O_2 and increasing concentrations of aqueous cinnamon extract and then spotted adjacently on YEPD agar plates.

RNA Extraction and Reverse Transcription

Total RNA was extracted using Lithium acetate and Trizol reagent. From samples containing approximately 10^7 cells, re-suspended in 200µl of lithium acetate and incubate at 50• C for 10 minutes to broken the cells and then add 1ml of Trizol reagent. RNA extraction was performed following the protocol provided by the manufacturer. The RNA concentration was determined by spectrophotometric analysis by read absorbance at 260 nm and 280 nm. The ratio of 260/280 of the all isolated RNA was in between 1.8-2.1. A maximal amount of Total RNA (5 µg) was then purified by treated with using RNase inhibitor and DNase I enzyme (Fermentas), following the protocol in the manufacturer's instructions. 1 µg of RNA was used for c-DNA synthesis using C-DNA kit (Applied Bioscience) according to manufacturer's instructions.

Gene Expression Analysis by Real-Time Quantitative Polymerase chain reaction (RT-PCR)

A list of genes described as involved in adaption and preadaptation to oxidative stress in *S. Cerevisiae* was reported by Anna et al (2011)[25]. Four genes (together with two housekeeping reference genes) were selected for real-time PCR expression Analysis; Table 1 shows genes chosen for this work, and metabolic functions and summarized in Gene Ontology terms listed in second column. The Real-Time PCR reactions for all samples were performed in a Light cycler (LC 480, Roche, USA). The kit for the Real-Time PCR containing master mix (SYBR green) purchased from Roche, USA. The reaction protocol for RT-PCR for all genes was followed as manufacturer instruction. For each gene, a standard curve was determined with yeast genomic DNA. Two housekeeping genes (ACT1, FBA1) have been amplified from all c-DNA samples. This allowed us to find the most stable genes for normalization. The housekeeping genes that resulted was 2 (stability cutoff, 0.15) and the stability of ACT1 and FBA1 was confirmed in all samples for both stressed and non-stressed conditions. The study was carried out using six Biological repetitions (independent yeast cultures), and each time real-time PCR was performed in two technical repetitions. Both experimental errors were finally considered in the global standard deviation.

Table 1: **PCR primers and gene selected for this study used for expression quantification (Anna et al, 2011) [24]**

gene	gene ontology terms	primer	sequence (5′-3′)	annealing temperature (̊C)	PCR product size (bp)
ACT1	cytoskeleton cell wall organization	ACT1-Qfw ACT1-Qrv	AATGCAAACCGCTGCTCAATCTTCTTCA AATACCGGCAGATTCCAAACCCAAAACAG	60.0	145
FBA1	glycolysis gluconeogenesis	FBA1-Qfw FBA1-Qrv	CTCCATTGCTGCTGCTTTCGGTAACTGT GAACCACCGTGGAAGACCAAGAACAATG	60.0	153
SOD1	response to stress superoxide dismutase activity	SOD1-Qfw SOD1-Qrv	GGTAACGTAAAGACGGACGAAAATGGT TTCAAAGATTCTTCAGTGTCACCCTTACCTA	60.0	155
CTA1	response to reactive oxygen species catalase activity	CTA1-Qfw CTA1-Qrv	CAGTACCACGGTTCCTTACCAAGAAGC CCATCTCTGATAGCGGGATTGAAAAAT	60.0	165
CTT1	response to reactive oxygen species catalase activity	CTT1-Qfw CTT1-Qrv	TGATTCCGTTCTACAAGCCAGACTTTTC GTATTGGGAATCACCTTTGGAGTATGGAC	60.4	136
GSH1	esponse to hydrogen peroxide glutathione biosynthesis	GSH1-Qfw GSH1-Qrv	TCTATGCTCGACGTTTGCCATGACAAGATAC TTCAAATATGGAGAAGCTGGTGTTGCCTCTAA	60.0	143

Statistical Analysis

Data were expressed as mean± SD. Significance of mean values of different parameters between the different groups were analyzed using one way analysis of variences (ANOVA) after ascertaining the homogeneity of variance between the treatments.

RESULTS

Total Phenolic contents

Total phenolic compounds of the cinnamon extract measured by Folin Ciocalteu method. The phenolic content of the cinnamon aqueous extract was found to be 481 mg GAE/100g (figure 1). Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics found in plants with known properties, which include free radical scavenging activity. The flavonoids content of aqueous cinnamon extract was 300 mg of TE/100gm (Figure 1).

Total Antioxidant Activity of Aqueous extract of cinnamon

In the present study total antioxidant capacity of aqueous cinnamon extract was measured using three different methods namely Ferric Reducing Antioxidant Power (FRAP) Assay, DPPH RSA and ABTS RSA. The results are presented graphically in figure 2. The total antioxidant capacity of aqueous cinnamon extract by FRAP, DPPH RSA and ABTS RSA was 451.47 mg, 1058.75 mg and 5341.704 mg of TE /100g respectively (Figure 2).

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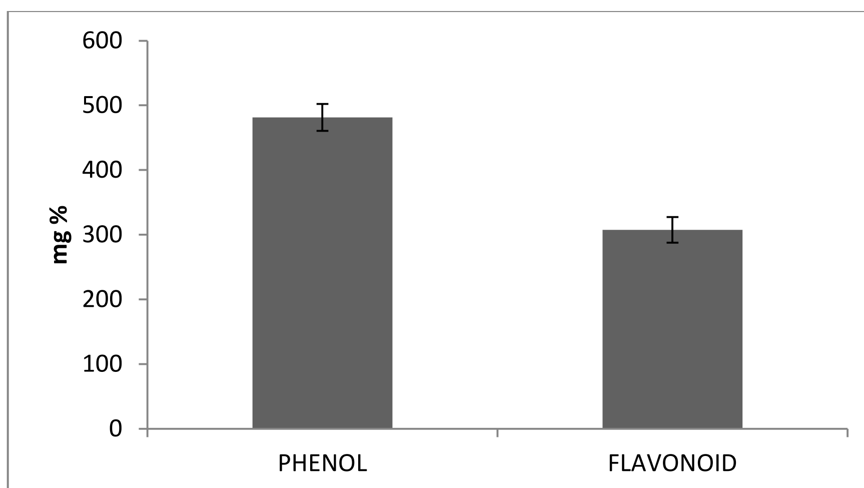


Figure 1: Total phenol and Flavonoid content of aqueous extract of cinnamon

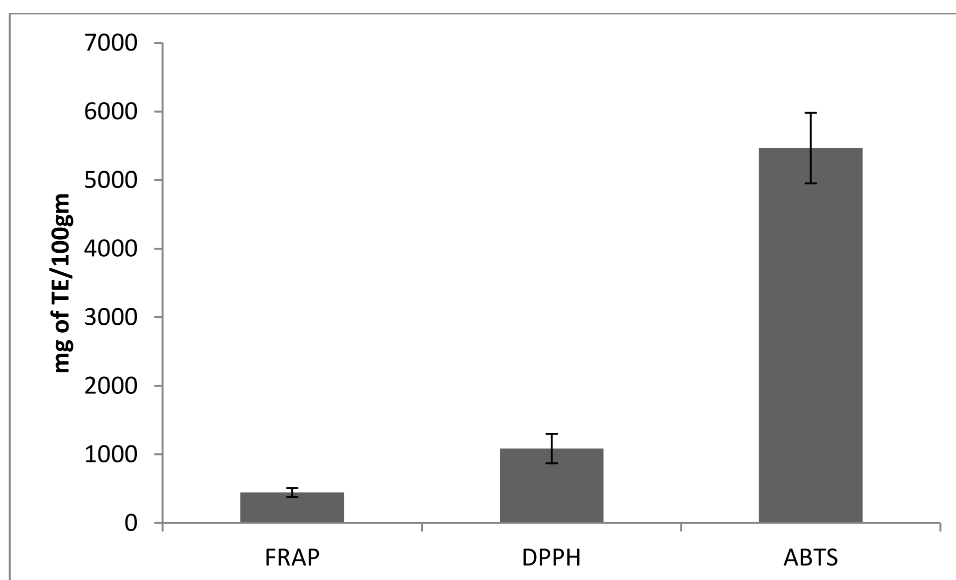


Figure 2: Free radical scavenging activity of Aqueous extract of cinnamon by FRAP Assay, DPPH RSA and ABTS RSA

Yeast Spot assay

To evaluate the growth of yeast cells in different treatments, spot assay technique was also used and the results obtained are presented in figure 3. The cinnamon extract added to the yeast cell was 0.2mg/ml and 0.4mg/ml of their phenolic compound. The growth of yeast cells did not show any adverse effect when yeast cells are grown with aqueous cinnamon extract. When H_2O_2 stress was given to yeast cells it showed a marked effect on growth. Addition of cinnamon extract at both the levels (0.2 mg/ml and 0.4mg/ml) showed marked protection against H_2O_2 stress.

Effect of Aqueous cinnamon extract on Antioxidant Enzyme gene expression in *Saccharomyces cerevisiae*

The gene expression pattern of antioxidative genes of *S. cerevisiae* cells was analysed by RT-PCR in control yeast cells (not exposed to H_2O_2), oxidative stress induced cells (10 mM H_2O_2 at logarithmic phase) and cells pretreated with cinnamon extract to yeast cells. In *S. cerevisiae* oxidative stress induce the expression of various defence genes. The Antioxidant enzymes induced by hydrogen peroxide in yeast cells are cytosolic CuZn-SOD (*SOD1*), mitochondrial Mn-SOD (*SOD2*), cytosolic catalase T (*CCT1*), catalase A (*CTA1*), γ-glutamylcysteine synthase (*GSH1*). Superoxide dismutases disproportionate superoxide anion to H_2O_2 and $O_2^{\bullet}$. In the present study, Yeast cells when exposed with 10 mM H_2O_2 showed a significant increase ($P \leq 0.05$) in

expression of *SOD1* (2.4 fold) compared to control while the cells pretreated with cinnamon extract (0.2mg/ml and 0.4mg/ml) showed significantly decrease in *SOD1* activity about 1.1 fold, as the phenolic compound is having protective mechanism over cinnamon extract against oxidative stress (figure 4B).The metabolically produced H_2O_2 within the cell is been removed by the action of catalase enzyme. In this study, Oxidative stress also noticed by up regulation of genes *CTT1* and *CTA1* (figure 4B, 4C). The expression profiles of these two genes were increased up to 1.6 and 2 fold in stressed cells, respectively. The expression pattern of *CTT1* and *CTA1* genes alters, when the cells are pretreated with the cinnamon extract and hence significantly decreases($P \leq 0.05$) the expression level same as control cells. Another *GSH1* gene was found to be inducible by H_2O_2. In the same manner as *GSH1* gene expression increased significantly in stressed cells while significantly decreased ($P<0.05$) in cinnamon extract treated cells (figure 4D).Therefore, Aqueous cinnamon extract had potent antioxidant compound that suppresses the oxidative stress.

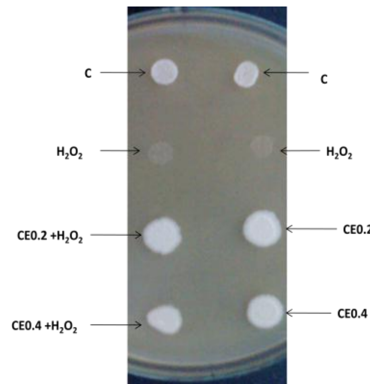
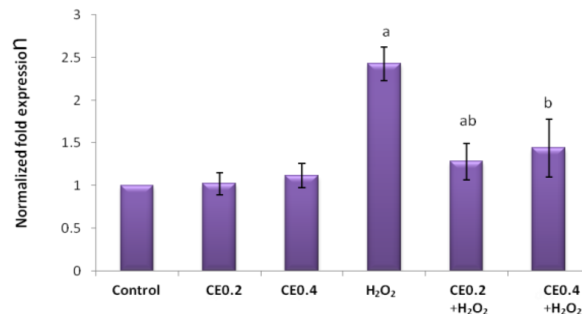
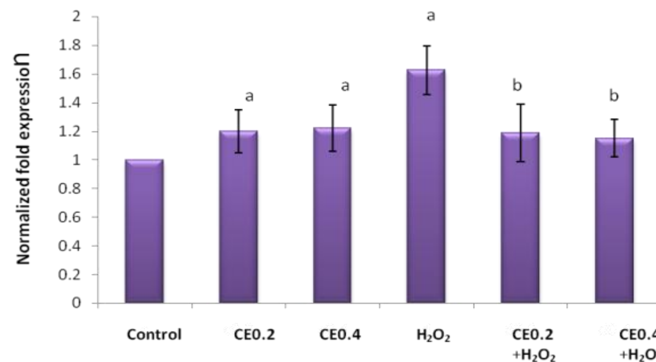


Figure 3: Patch assay of yeast cells with aqueous cinnamon extract

4A

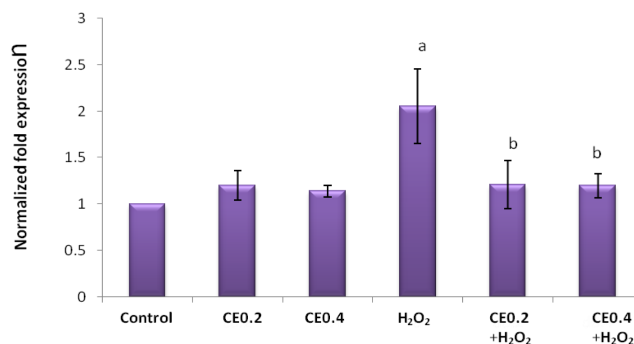


4B



4C

CTA Activity



4D

GSH Activity

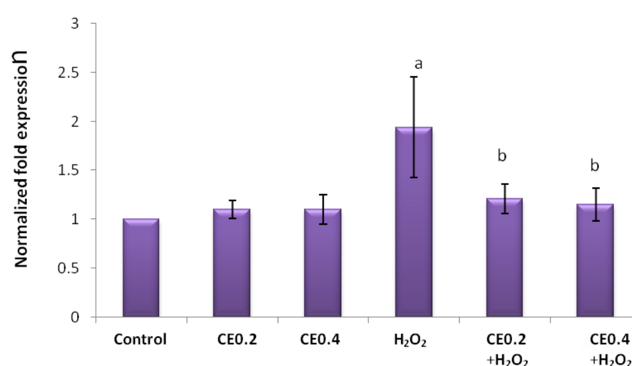


Figure 4: Expression levels of SOD, CTT1,CTA1 and GSH1 in *S.cerevisiae* cells. The control sample was not treated with aqueous cinnamon extract or H_2O_2. The sample treated with two different concentrations of cinnamon extract (0.2mg/ml and 0.4mg/ml). For each data set, the control was selected as reference (expression level=1).Error bars show standard deviations. The relative normalized fold expression is calculated by the ΔΔCt method using ACT1 and FBA1 as reference gene. Data are presented mean ± SD [a] indicates significant difference from control [b] indicates significantly different from H_2O_2 at P< 0.05. Values represent the mean of two replication of six different biological samples.

DISCUSSION

In the present study, we had demonstrated the total phenolic content, total flavonoids and the total antioxidant capacity of *cinnamomum zeylanicum* by different free radical scavenging methods such as FRAP, DPPH RSA and ABTS RSA *in vitro* and *in vivo* experimental system using Yeast model. Phenolics compounds such as flavonoids, phenolic acid and tannins posses diverse biological activities. These activities might be related to their antioxidant activity [26]. The total phenolic and flavonoid content of aqueous cinnamon extract shown in figure 1. Diksa Devi (2013) reported higher amount of total phenol and flavanoid content of Aqueous cinnamon extract compared to our result [27].It may be due to difference in extraction procedure. Diana Chenga (2012) also reported that CE contained 441 GAE/g total phenols [28]. Aqueous cinnamon extract possessed specific bioactive compound such as chlorogenic acid, t-cinnamic acid, cinnamic acid methyl ester, cinnamide, cinnamyl alcohol, clove oil, p-coumaric acid, o-coumaric acid, curcumin and eugenol have insulin enhancing activity and also function as antioxidant [29]. The phenolic and flavonoids compounds of cinnamon was different with their different variety and extraction solvent [13].DPPḢ$^{\bullet+}$ and ABTS$^{\bullet+}$ radicals are among the most popular spectrophotometric methods for determination of the antioxidant capacity of food, beverages and vegetable extracts. Both the chromogen radical compounds can directly react with antioxidants. Additionally, DPPḢ$^{\bullet+}$ and ABTS$^{\bullet+}$ scavenging methods have been used to evaluate the antioxidant activity of compounds due to their simple, rapid, sensitive, and reproducible procedures. In the present work, cinnamon extract showed the higher free radical scavenging capacity (figure.1 and 2). The inhibitory effect of cinnamon

extract against free radicals showed that the cinnamon had the antioxidant compound which played a major role against oxidative stress. The cinnamon extracts analyzed in this study possessed very strong antioxidant properties. These properties have been previously demonstrated in organic or aqueous extracts, in different species and with various assays [30-32].

Yeast have been serving as important model systems to understand how eukaryotic cells sense and respond to oxidative stress. Aerobic organisms are constantly exposed to ROS as generated by normal metabolism especially by respiration. There are several antioxidant systems with an apparent functional redundancy, which may provide an evolutionary advantage. In addition, it has been previously demonstrated the *S. cerevisiae* and *C. albicans* possess and adaptive oxidant stress response, whereby initial exposure to non-lethal dose of H_2O_2 or $O_2^{\bullet}$ generating confers resistance to further severe and normally lethal dose of same oxidant [33-37]. The yeast *Saccharomyces cerevisiae*, is well known example to elicit different adaptative stress response mechanisms in an effort to survive and thrive in hostile conditions. In yeast, the ability to withstand a lethal oxidative stress depends on the enhanced expression of specific genes which encode for antioxidant enzymes with both protecting and repairing role [38-39]. Yeast treated with H_2O_2 produced oxidative stress, cause variation in the activities of various enzymes and antioxidant molecules. H_2O_2 is ubiquitous molecule formed as a product of various oxidases and by auto-oxidation of haemoproteins and flavoproteins [40] So that it must detoxified in to cell can lead to highly reactive hydroxyl radical. Detoxification of H_2O_2 is mediated by catalases, which convert H_2O_2 to O_2 and H_2O and by glutathione peroxidises which use GSH as reluctant for the breakdown of peroxidises. *Saccharomyces cerevisiae* cells possessed multiple H_2O_2 detoxifying enzymes, such as catalases, cytochrome-c peroxidase, glutathione peroxidases, glutaredoxins and peroxiredoxins. Different amount of H_2O_2 have distinct effects on antioxidative enzyme. for example while low H_2O_2 concentrations (0.5mM) induced catalase, GR, SOD activity while at high H_2O_2 levels (50mM) induced catalase and GR activity but strongly inhibited SOD activity [41].We have also evaluated the antioxidant potential of cinnamon extract in vitro as well as in vivo using Yeast as model. To address whether the cinnamon extract can serve as external antioxidant source in the yeast, treated with 10 mM H_2O_2 ; cinnamon extract at two concentrations (0.2mg/ml and 0.4mg/ml). The spot array indicated that exogenous cinnamon extract reduced oxidative stress in *S. cerevisie* (figure3). These results demonstrated that yeast strain can be used extracellular cinnamon extract to reduce the cell death. Moreover it can reduce intracellular oxidative stress induced by H_2O_2. Superoxide dismutases (SOD) are very important metallo-enzymes involved in cellular protection against superoxide ($O2^{\bullet -}$) toxicity. Superoxide dismutases disproportionate superoxide anion to H_2O_2 and O2. The Cu/ZnSOD appears to be the major enzyme involved in removing superoxide anions from the cytoplasm and possibly also the peroxisome [42].In the present study, there is an increase in total SOD activity (2.4 fold) was observed in cells which were subjected to mild oxidative stress (10 mM H_2O_2) and these increased higher than the control cells (figure 4A).Anna lante (2011) also observed that yeast cells when exposed to H_2O_2 clearly up regulated *SOD1* ,Even when yeast cells are pre-treated with red chicory that significantly decreased the SOD1 expression [25]. In our study, cinnamon extract treated cells also sensed the lower stress at both concentrations (0.2mg/ml and 0.4mg/ml) and significantly decreased the SOD1 expression. *S. cerevisiae* has catalase A and catalase T, encoded by the *CTA1* and *CTT1* genes, respectively. [43-44]We addressed the both the isoform that protects cells against H_2O_2. In our study, both *CTT1* and *CTA1* expression significantly increased upto 1.6 and 2 fold in H_2O_2 treated cells, respectively. These result indicated that *CTA1* play major role to reduced oxidative stress in yeast cell. Dorval martins (2014) reported the increased gene expression of *CTA1* and *CTT1* in *S. cerevisie* cells expressed to H_2O_2 of very low concentration, similar results are also observed in the present study [45].The CE pretreated yeast cells showed significantly decreased expression levels of both genes *CTA1* and *CTT1*, as non treated cells at both concentrations. These results indicate that CE possesses the various phenolic compounds which act as antioxidants and scavenge free radicals that lowered the oxidative stress at molecular level. Many study reported that cinnamon extract possessed antioxidant activity in rat model [46-47]. There was no data available on cinnamon extract reduced oxidative stress in yeast model and antioxidative gene expression. A previous study demonstrated that procyanidins and catachine, a pure antioxidant molecule affect the *CTT1* gene [48].The genes involved in GSH biosynthesis have been identified in *S. Cerevisiae* are *GSH1* and *GSH2*. *GSH1* gene was found high copy assessor of mutation in a gene encoding mitochondrial protein that suggesting GSH plays important role in protecting the mitochondria from oxidants. In this study, *GSH1* was also affected by oxidative stress and upregualated the expression upto 2 folds. Dani et al (2008) [48] also reported pure molecule of Resveratrols molecule specific for *GSH1* gene.

CONCLUSION

In summary, this work provide the new evidence concerning the mechanism by which H_2O_2 changes the antioxiidative enzyme gene expression in yeast model and the cinnamon extract which possess the various antioxidant compound which regulates the oxidative stress at molecular level at both concentrations (0.2mg/ml and 0.4mg/ml). Further study is for required to be done upon on cinnamon extract for which specific compound are actually responsible regulating the antioxidative gene expression in yeast.

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Abbreviations

ROS : Reactive Oxygen species
DPPH : 1, 1-diphenyl- 2-picryl-hydrazyl
DPPH RSA: DPPH Radical Scavenging Ability;
ABTS : 2, 2 azinobis (3eethylbenzothiazoline-6-sulfonic acid) diammonium salt;
ABTS RSA: ABTS Radical Scavenging Ability;
FRAP : Ferric Reducing Antioxidant Power Assay
TEAC : Trolox Equivalent Antioxidant Capacity
Trolox : 6- hydroxy-578-tetra methyl-chromane-2 carboxylic acid.
RT-PCR : real Time Polymarase Chain Reaction
BHT : Butylated Hydroxytoluene
TPTZ : 2, 4, 6-tris (2-pyridyl) -s- triazine
YEPD : Yeast extracts potato dextrose
PBS : Phosphate Buffer saline
RNA : Ribonucleic acid
c- DNA : Complementary Deoxyribonucleic acid
SOD : Super oxide Dismutase
CAT : Catalase
GSH : Glutathione

REFERENCES

[1] Murphy MP. J Biochem 2009 ;417 1–13.
[2] Chance B, Sies H and Boveris A. Physiol Rev 1979;59:527–605.
[3] DJ Jamieson. Yeast 1998;14:1511–1527
[4] Shull S, et al. J Bio. Chem 1991; 266:24398–24403.
[5] Ho Y S, Dey MS, Crapo JD. Am J Physiol 1996; 270:810– L818.
[6] Rohrdanz E, Kahl R. Free Radic Biol Med 1998; 24:27–38.
[7] Franco AA, Odom RS, Rando TA. Free Radic Biol Med 1999 ; 27: 1122–1132.
[8] Fuping Lu, Yu wang, Dongquing bai, Lianxiang Du. Proc Biochem 2005; 40:3614-3618.
[9] V Prasanna And S Sreelatha. Int J Pharm Bio Sci 2014; 5 (2): 167 – 177.
[10] Ooi LSM, Li Y, Kam SL, Wang H, Wong EYL, & Ooi VEC. The American J Chinese Med 2006; 34(3), 511–522.
[11] Kwon HK, Hwang JS, So JS, Lee CG, Sahoo A, Ryu JH, et al. BMC Cancer 2010;10:392–401.
[12] Qin B, Panickar KS & Anderson RA. J Diab Sci Technol 2010; 4(3): 685–693.
[13] J Mancini-Filho, et al. Bollettino Chimico Farmaceutico 1998; 137(11):443–447
[14] GK Jayaprakasha and LJM Rao. Crit Rev Food Sci Nutr 2011; 51(6): 547–562.
[15] Lin C.-C., S.-J. Wu, C.-H. Chang, and L.-T. Ng. Phytother Res 2003; 17(7):726–730.
[16] H Cao, MM Polansky, and RA Anderson. Arch Biochem Biophy 2007; 459(2): 214–222.
[17] Lee S.-C., W.-X. Xu, L.-Y. Lin, J.J.. Yang, and C.-T. Liu. J Agr Food Chem 2013; 61(20):4905–491.
[18] Kim SH, SH Hyun, and SY Choung. J Ethnopharmacol 2006; 104, 1(2):119–123.
[19] Khan A, et al. Diabetes Care 2003; 26(12): 3215– 3218.
[20] Singleton VL, R Orthofer and RM Lamuela-Raventos. Enzymol 1999; 299:152-178.
[21] Zhishen J, Mengcheng T, Jianming. Food Chem 1999;64: 555-559.
[22] Benzie IFF, Strain JJ. Meth Enzymol 1999; 299, 15–27.
[23] Brand-Williams W, Cuvelier ME, & Berset C Lebensm. Wiss. Technol 1995; 28: 25–30.

[24] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Free Radic Biol 1999;26(9-10):1231-7.
[25] A Lante, Tiziana Nardi, Federico Zocca, Alessio Giacomini, and Viviana Corich. J Agric Food Chem 2011;59:5318–5324.
[26] Tsai TH, Tai TH, Chien YC, Lee CW, Tsai O. J Food Chem 2008;110:859–864.
[27] DD Tacouri, Deena Ramful-Baboolall, Daneshwar Puchooa. Asian Pac J Trop Dis 2013; 3(4):253-261.
[28] DM Chen, et al. Food Chem 2012; 135(4): 2994–3002.
[29] Anderson RA, et al. J Agric Food Chem 2004; 52: 65−70
[30] Tourin˜oS, et al. J Agric Food Chem 2005; 53:4728–4735.
[31] Kim HY, Kim K. J Agric Food Chem 2003; 51:1586–1591.
[32] Shan B, Cai YZ, Sun M, Corke. J Agric Food Chem 2005; 53: 7749–7759.
[33] Collinson LP, Dawes IW. J Gen Microbiol 1992; 138: 329–335.
[34] Jamieson DJ. Yeast 1998; 14:1511–1527.
[35] Jamieson DJ, Rivers SL, Stephen DW. Microbiol 1994; 140: 3277–3283.
[36] Flattery-O'Brien J, Collinson LP, Davies IW. . J Gen Microbiol 1993; 139: 501–507.
[37] Alvarez-Peral FJ, Zaragoza O, Pedre˜no Y, Arg¨uelles JC. Microbiology 2002; 148: 2599–2606.
[38] Davies JM, Lowry CV, Davies KJ. Arch Biochem Biophys 1995; 20: 1–6.
[39] Estruch F. FEMS Microbiol Rev 2000; 24: 469–486.
[40] Grant CM, Maclever FH & Dawes I. Curr Genet 1996; 29:511-515.
[41] P Gonz´alez-P´arraga, Jos´e A Hern´andez and Juan Carlos Arg¨uelles. Yeast; 2003; 20: 1161–1169.
[42] Gralla EB and Valentine JS. J Bacteriol 1991; 173:5918–5920.
[43] Cohen, G., Rapatz, W. and Ruis, H.. Eur. J. Biochem. 1988; 176: 159–163.
[44] Hartig, A. and Ruis, H. Eur. J. Biochem. 1986; 160: 487–490.
[45] D Martins, Ann M.English ,Redox Biolog 2014; 2308–313.
[46] SS Moselhy And Husein KH Ali. Biol Res 2009; 42: 93-98.
[47] Suganthi R, S Rajamani, MK Ravichandran, and CV Anuradha. J Med Food 2007; 10(1): 149–153.
[48] Dani C et al. J Agric Food Chem 2008; 56, 4268–4272.