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# Antioxidant and Anticancer activities of yeast grown on commercial media

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## ABSTRACT

The use of Synthetic antioxidants is being widely restricted because of their reported toxic and carcinogenic effects. Thus, now there is considerable interest being focused towards finding antioxidants from natural sources with any unlikely effects. There is also considerable evidence which indicates lower risk of Cancer in yeast extracts and the used commercial media. The present study was conducted to determine antioxidant activity of yeast extracts grown on four different commercial media using DPPH, total phenolic content, total antioxidant activity and TBARS and to study the anticancer activity of yeast extracts on the mentioned media against human hepato carcinoma cell line (HEPG-2) using the MTT assay, Cell Cycle Analysis and mRNA Expression of Cell Apoptosis-related Gene. The yeast extract of corn meal was found to have more antioxidant activity than other commercial media. The antioxidant potential was then correlated with the total phenol content. The yeast extract of corn meal showed maximum anticancer activity against HEPG-2 as compared to that of other used media. Overall, the results achieved in this study are promising and indicate that yeast fermentation on corn meal medium is better than other used media to get on antioxidant and anticancer activities.
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**Keywords**: Yeast, commercial media, Antioxidant, Anticancer activity.

## INTRODUCTION

Yeasts have a positive image with consumers, as they are considered a safe source of ingredients and additives for food processing (Boze et al., 1992; Bekatorou et al., 2006; Tsunatu et al., 2017) Preparations of baker's and brewer's yeasts have been available for many years as dietary, nutrient supplements because of their high contents of B vitamins, proteins, peptides, amino acids and trace minerals. Also, yeasts are often considered as an alternative source of protein for human consumption (Buzzini et al., 2005; Chaucheyras-Durand et al., 2008; Pienaar et al., 2012).

Many products are now derived from yeasts and, according to Abbas (2006), about 15–20% of the global industrial production of yeasts is used for this purpose. The production of antioxidants, aromas, flavors, colors and vitamins by yeasts. Other detailed accounts of these topics may be found in (Halasz and Lasztity, 1990; Türker, 2014).

The role of antioxidants in nutrition and health, as well as their mechanisms of action, have been extensively researched

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(Serafini et al., 2011). Although the biological functions of polyphenols and/or metabolism in the human body are not completely established, there is a consensus that the antioxidant activity of flavonoids could be a combination of metal chelating properties and free radical scavengers (Bravo, 1998).

In contrast to their ability of reducing food digestibility caused by the potential phenolics to bind and precipitate macromolecules; interest in food phenolics has increased, because of their antioxidant and free radical scavenging abilities (Lugasi and Hovari, 2003), metal chelators and enzyme modulators (Dulger et al., 2002). Many phenolics can exhibit antioxidant activity as their extensive, conjugated π-electron systems allow ready donation of electrons, or hydrogen atoms, from the hydroxyl moieties to free radicals. However, the antioxidant efficacy, in terms of reaction stoichiometry and reaction kinetics may vary considerably (Lugasi and Hovari, 2003). This is dependent on structural features, such as the number and positions of the hydroxyl moieties on the ring systems, and the extent by which the unpaired electron in the oxidized phenolic intermediate can delocalise throughout the molecule. Thus, most phenolics, especially flavonoids are very effective scavengers of hydroxyl and peroxyl radicals. Phenolics are chelators of metals and inhibit the Fenton and Haber-Weiss reactions abilities (Lugasi and Hovari, 2003; Dulger et al., 2002), which are important sources of active oxygen radicals. On account of their antioxidant effects, phenolic compounds inhibit the development of cancerous tumours, reduce a risk for cardiovascular disease, and have showed anti-bacterial, anti-inflammatory, anti-spasmodic and anti-diarrheic properties (Abdoul-latif et al., 2012)

Fermentation is a good technology with great potential for application on the production or extraction of antioxidant active compounds from natural sources. New bioactive compounds could be found during fermentation. Moreover, modification of fermentation process could be tailored so as to increase the bio accessibility of bioactive compounds. Production of bioactive compounds yet remains a quite unexplored potential, which could be accomplished by utilizing new fermentation process. Therefore, in the future, it can be anticipated that fermentation could be used to design food with health effects. Some fermentation processes are available on the applications of production of antioxidant activity compounds. However, the underlying mechanisms affecting antioxidative activity during fermentation are varied, and the production of antioxidant activity compounds during fermentation (Hur et al., 2014).

Some of the most compelling evidence of a protective effect of diets against cancer, in recent years, is the evidence on the intake of fruits and vegetables (Block et al., 1992; Fokou et al., 2017). EPIC (European Prospective Investigation into Cancer and Nutrition) is an important study that indicates that these retrospectively obtained results, at least respecting to cancer, might have been somewhat overestimated, however, still a significant reduction of consumption of fruits and vegetables on e.g. colorectal cancer was found (Bouayed and Bohn, 2010). Polyphenols can further act by inhibiting cell proliferation, which is deregulated in cancer. This inhibition has been demonstrated *in vitro* in many tumor cell lines. Although the antiproliferative effects of polyphenols in general and in particular of flavonoids and isoflavonoids in cell cultures seems well established, there are relatively few data regarding the *in vivo* antiproliferative activity, and virtually nothing is known about the clinical relevance of this bioactivity (Birt et al., 2001). This anti-proliferative effect suggests that polyphenols may have an effect via regulating the cell cycle or inducing apoptosis in tumor cells. In fact, many studies have shown the effect of polyphenols on the cell cycle of tumor cells in cultures in *in vitro* assays.

So, in the presented study, we make differentiation between the antioxidants which

produced due to the dry yeast fermentation on four commercial media to get on the better one.

## MATERIALS AND METHODS

All represented work was done in Microbiology and Biology Labs of National Organization of Drugs Control and Researches (NODCAR).

### Media preparation

Three commercial media were prepared by weighting of 30 grams and cooked on hot plate then filtrated by cotton and sterilized in the autoclave at 121 °C for 20 min. while basal medium was prepared according to Chen et al. (2010), yeast-peptone-dextrose (YPD) broth containing 0.5% (w⁄v) yeast extract, 1% peptone and 2% glucose by the same conditions of sterilization according to Ausubel et al. (1994).

### Culture of commercial yeast on different used media

1 gram of active dry yeast baking-Helew El-Shame (*Saccharomyces cerevisiae*) was added on 100 ml broth of each sterilized media, shaking and incubated at 37 °C for 48 hours (Zakpaa et al., 2009).

### Extraction of the secondary metabolites from cultivated yeast media

Each medium was centrifuged by using cooling centrifuge at 4 °C, 10,000rpm for 20 min to get on the clear supernatant, and kept in the refrigerator up to use (Chen et al., 2010).

### Lyophilization of obtained products

All produced supernatant was lyophilized by using of Edwards Freeze Dryer Modulyo instrument -frezze drier lab -Cairo University to get on lyophilized powder(Gaidhani et al., 1967; List, 1967).

### Antioxidant activity of the extracellular secondary metabolites

#### *DPPH 2, 2, -diphenyl-1- picrylhydrazyl radical scavenging ability*

Scavenging effect was determined according to the method of (Shimada et al., 1992). Four dilutions of each extract were mixed (1:1) with methanolic solution containing DPPH radicals, resulting in a final volume of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 minutes in the dark, with absorbance then measured at $\lambda_{517}$nm. A blank methanolic solution containing DPPH radicals without test compound was used as a negative. Gallic acid was used as comparative positive control. The scavenging ability (%) was calculated using the following equation:

$[(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] \times 100$

Radical scavenging activity was expressed as the concentration (mg $mL^{-1}$) that scavenged 50% of the DPPH radicals ($IC_{50}$) and was obtained by interpolation from linear regression analysis of each extract (0.1 – 50 mg $mL^{-1}$). Three replicates for each concentration were used for the determination of the mean DPPH values of yeast extracts samples.

#### *Total phenolic content (TPC)*

Total phenolic content of yeast extracts obtained from each used nutritional medium was estimated using the Folin-Ciocalteu reagent method (Kahkonen et al., 1999; Ainsworth and Gillespie, 2007). 1ml of each Sample extracts were mixed with 250 µL of 10% (v/v) Folin-Ciocalteu reagent, followed with the addition of 500 µL saturated sodium carbonate (10%, w/v aqueous solution) after 2 minutes of incubation at room temperature. The mixture was placed in the dark for 1 hour. Absorbance was then measured at λ750nm. The concentration of total phenols was calculated based on a calibration curve using gallic acid. The phenol content was expressed as gallic acid equivalent (GAE), which reflects the phenol content, as the amount of gallic acid units in 1 gram of extract (mg GAE

$g^{-1}$). Three replicates were used for the determination of the mean total phenolic content values of the yeast extracts samples.

***Total antioxidant capacity (TAC) phosphomolybdenum method***

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method according to the procedure described by (Prieto et al., 1999). A 0.3 mL of extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 ºC for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (UVmini-1240) against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) with methane

***Thiobarbituric acid substances (TBARS)***

One milliliter of yeast extracts, 0.2 mL of 8.1% (w / v) sodium dodecyl sulfate, 1.5 mL of acetic acid and 1.5 mL of 0.5% of TBA were mixed and heated in a water bath at 95° C for 60 min. After cooling, 5 mL of n-butanol / pyridine (15:1v / v) was added and agitated vigorously. The absorbance of the organic layer was read at 532 nm in a spectrophotometer after centrifuged at 4000 g for 10 min. The 1, 1`, 3, 3`-tetramethoxypropane was used as standard and the level of lipid peroxidation was expressed as nanomoles of malondialdehyde (MDA) per milligram of tissue protein (Zhang et al., 2008).

**Cytotoxicity (MTT Assay)**

Cytotoxic effects of two comparable yeast products were tested against HepG2 in vitro cell lines by the MTT assay as previously described by Alley et al. (1988); Aquino et al. (1989) and El Sharkawi et al. (2015). human hepatocellular carcinoma cells (HepG2 ATCC number HB-8065) were propagated in 75 $cm^2$ cell culture flasks using DMEM (GIBCO-USA) supplemented with 10% (v/v) fetal bovine serum (GIBCO-USA) and incubated in 5% (v/v) $CO_2$ incubator at a temperature of 37°C.Confluent cells were detached using 0.25% (w/v) trypsin solution and 0.05% (v/v) EDTA (GIBCO-USA) for 5 min. Detached cells were cold centrifuged using (Jouan-Ki-22- France). Cell pellets were resuspended in growth media. Two comparable yeast products were inoculated at concentrations of 0.005, 0.01, 0.02,0.04, 0.08, 0.16, 0.32, 0.64, 1.25,2.5, 5.0, 10 mg/l in 96-well cell culture plates(TPP-Swiss), plated were incubated at 37°C for 24 hours(Jouan-France) till confluency. Dead cells were washed out using phosphate-buffered-saline (PBS) and 50 µl of MTT (Serva- Swiss) stock solution (0.5 mg/ml) were added to each well. Four hours later of incubation at 37°C, the supernatants were discarded and the developed formazan crystals were solubilized using 50 µl per well of dimethyl sulfoxide (DMSO). Plates were incubated in the dark for 30 min at 37°C and absorbance was determined at a wavelength of 570 nm using micro plate reader (ELX -800, Biotek - USA). Cell viability percentage was calculated using the following formula:

Cell viability (%) = OD of treated wells x 100/OD of control wells

The cell viability % was plotted against the tested two yeast products concentrations.

The $IC_{50}$ values of test comparable yeast products were determined using Masterplex 2010 hitachia (GIRSS) software program. The effect of each yeast product as anticancer agent was examined by evaluating the cytotoxic potential of produced products in HepG2 cells pretreated with 25 and 50 µg for 24 hrs. Morphological alterations of cells were analyzed using an inverted microscope (Nikon-Japan).

**Anticancer activity**

***Cell Cycle Analysis***

HepG2 cells pre-cultured in 25 $cm^2$ cell culture flasks were treated with the $IC_{50}$ values of two comparable yeast products for 24hrs. For cell cycle analysis, the detached cells were harvested and fixed gently with 70% (v/v) Methanol and kept at +4 °C. Cells were re-suspended in PBS containing 40 µg/ml PI and 0.1 mg/ml RNase and 0.1% (v/v) Triton X- 100 in a dark room. After 30 min at 37 °C, the cells were analyzed using the flow-cytometer (Becton-Dickinson, San Jose, CA, USA) equipped with an argon ion laser at a wavelength of 488 nm. The cell cycle and sub-G1 group were determined and analyzed, as described previously.

***mRNA Expression of Cell Apoptosis-related Genes***

Total RNA was extracted from control and treated HepG-2 cells using the Gene JET RNA Purification kit (Fermantus-UK) according to the manufacturer's protocol. The concentration and the integrity of RNA were assessed spectrophotometrically at 260/280 nm ratio and by gel electrophoresis, respectively. First-strand cDNA was synthesized with 1 µg of total RNA using a Quantitect Reverse Transcription kit (Qiagen, Germany) in accordance with the manufacturer's instructions. These samples were subsequently frozen at a temperature of -80°C until use for determination of the expression levels of Bax and BCl- 2 genes using real-time PCR. Quantitative real-time PCR was performed on a Rotor-Gene Q cycler (Qiagen, Germany) using QuantiTect SYBR Green PCR kits (Qiagen, Germany) and forward and reverse primers for each gene. The nucleic acid sequences of the primers were as follows:

Bax F 5'-GTTTCA TCC AGG ATC GAG CAG-3'Bax R 5'-CATCTT CTT CCA GAT GGT GA-3'bcl-2 F 5'-CCTGTG GAT GAC TGA GTA CC-3'bcl-2 R 5'-GAGACA GCC AGG AGA AAT CA-3'compared to the house keeping gene $\beta$-actin as a control β-actin F 5'-GTGACATCCACACCCAGAGG-3' β-actin R 5'-ACAGGATGTCAAAACTGCCC-3'.Real-time PCR mixture consisted of 12.5 µL 2x SYBR Green PCR Master Mix, 1 $\mu$L of each primer (10pmol/µl), 2 µL cDNA and 8.5 µLRnase-free water in a total volume of 25µL. Amplification conditions and cycle counts were a temperature of 95 °C for 15 min for the initial activation, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. Melting curves were performed after real-time PCR to demonstrate the specific amplification of single products of interest. A standard curve assay was performed to determine the amplification efficiency of the primers used. Relative fold changes. Expression of target genes (Bax and Bcl-2) were accomplished using the comparative $2^{-\Delta\Delta Ct}$ method with the $\beta$-actin gene as a house keeping gene to normalize the level of target gene expression. ΔΔCT is the difference between the mean ΔCT (treatment group) and mean ΔCT (control group), where ΔCT is the difference between the mean CT gene of interest and the mean CT internal control gene in each sample. Logarithmic transformation was performed on fold change values before being statistically analyzed, using the fold change values of three replicates for each gene.

***Apoptotic evaluation***

RNA was extracted from 24 hr treated cells with two comparable yeast products, treated and untreated control cells by using RNA isolation kit according to manufacturer's protocol. Extracted RNA was reverse transcripted to cDNA using Maxima first strand cDNA synthesis Kit. The mRNA expression of pro-apoptotic gene (Bax) and anti-apoptotic gene (Bcl-2) was carried out using the newly synthesized cDNA as template for PCR. Semi-quantitative RT-PCR was carried out according to Marone.2001 Using the specific primer for each gene (in a concentration of 10pmole/µl for each primer) and housekeeping gene (GAPDH). Gel

electrophoresis for PCR products (10µl) was carried out on 1.5% agarose gel using DNA ladder 1.5 KB and visualized using UV transillumiator after staining with ethidium bromide followed by denstiometric analysis of bands intensities which expressed as relative absorbance units. Data representing mRNA expression levels of Bax and Bcl-2 were calculated as band intensities compared to GAPDH by using alpha gel for rapid cell fixation and dispersion, the cell suspensions were added drop wise to 1 ml of cold 70% ethanol and incubated on ice for 45 min. After centrifugation, the cells were resuspended in 1ml of propidium iodide (PI) master mix (PBS containing 100 mg/ml RNase A and 40 mg/ml PI) and incubated in the dark at 37 ºC for 30 min. The various cell cycle phases were monitored using a FACSCalibur_ flow - cytometer (BD Biosciences, San Jose, CA). Cells were excited at 488 nm with an argon laser, and the emission from ten thousand cells was recorded using a 580-nm band-pass filter (FL2-H) 27. The obtained cell cycle profiles were analyzed using Cell Quest version 3.2 and Win MDI version 2.8 software's.

**Statistical analysis**

Experimental values were given as means ± standard deviation (SD). Statistical significance of data was analyzed at $P \leq 0.05$ (prism iPad graph) version 5.

## RESULTS

**Antioxidant activity of the extracellular secondary metabolites**

***DPPH***

The results of scavenging effect on DPPH radical by extracts of yeast products on four used mediawere showed in Figure 1, by measuring absorbance decrease at 517 nm. The scavenging activity of yeast metabolites in corn meal extract was significantly higher than those corresponding samples of yeast metabolites of other three media(p=0.0162). The results of basal medium, corn meal, oat meal and sugar cane sucker were: 44.27, 55.61, 33.32 and 26.02%, respectively

While, $IC_{50}$ values were illustrated in Figure.2 which determined for each extract of yeast products on four used media, the value of corn meal was significantly higher than other corresponding extracts (p=0.0071): 3.23 mg/ml while in basal medium, oat meal medium and sugar cane sucker were 2.19,1.83 and 1.87 mg/ml; respectively.

***Total Phenolic content (TPC)***

Present study entailed that corn meal yeast extract was significantly higher than other corresponding yeast extracts from used media (p=0.0117). The total phenolic content was estimated by Folin-Ciocalteu method and gallic acid was used as the standard reference (Figure 3).

Results of basal medium, corn meal, oat meal and sugar cane sucker were: 1387, 1990, 1129 and 982 µg gallic acid, respectively.

***Total antioxidant capacity (TAC) (phosphomolybdenum method)***

The Phosphomolybdenum reduction assay was based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature. Figure 3 was illustrated that total antioxidant capacity of yeast extract produced by corn meal is significantly higher than yeast extracts of other corresponding used media. Results were presented as following: basal medium 419, corn meal 521, oat meal 290and sugar cane sucker 141 µg/ml; respectively.

***Thiobarbituric acid substances (TBARS)***

In the present study, TBARS values of yeast extract by corn meal was significantly lower than yeast extracts by other corresponding media (Figure 1) data were basal medium, corn meal, oat meal and sugar cane sucker: 46.6, 18.04, 53.48and 70.34%, respectively.

***Cytotoxicity by MTT assay***

In the present study, the anti-proliferative potential of two comparable yeast extracts was conducted and it was noticed that yeast extract of basal medium showed a significant toxic effect than yeast extract produced by corn meal in HepG2 ($P<0.05$) **(**Figure 4**)**. Also, The $IC_{50}$ value was significantly decreased in case of basal medium yeast extract (433 mg/ml) while increased in case of corn meal (966 mg/ml) ($P<0.05$) in HepG2 as well ($P<0.05$) **(**Figure 5). The cytotoxic effect of basal medium and corn meal showed morphological change in the treated cells including cell membrane blebing and mitochondrial disruption. Also, it was noticed that the viability was cell type and concentration dependent.

**Anticancer activity**

***Cell Cycle Analysis***

The anticancer potential there was a noticed-up regulation of pro-apoptotic gene (Bax) accompanied with down regulation of anti-apoptotic gene (Bcl-2). The cell cycle analysis showed a cell cycle arrest at the G2/M phase after treating the cells with yeast extract of basal medium and/or corn meal. HepG2 cells were arrested with a percentage of 63.8% and 58.56% corn meal and basal medium respectively.

These results show that yeast extracts by corn meal and /or basal medium have enhanced the G2/M cell cycle checkpoint and as a result the cell arrest at this point has increased which enhances apoptosis and this proves the potential of yeast extracts as an anti-proliferative agent.

***mRNA Expression of Cell Apoptosis-related Genes***

The RT-PCR analysis for BAX, and BCL-2 genes has showed an upregulation in BAX which is a pro-apoptotic gene (basal medium/corn meal: 6.88/4.862 folds for HepG2) on the other hand; the results showed a down regulation in the BCl-2 gene which is an anti-apoptotic gene (basal medium/corn meal: 0.0796/0.211 folds for HepG2). These results positively point out an increased apoptotic activity in the treated cancer cell lines which supports the promising anti-cancer/proliferative potential of yeast extract of corn meal.

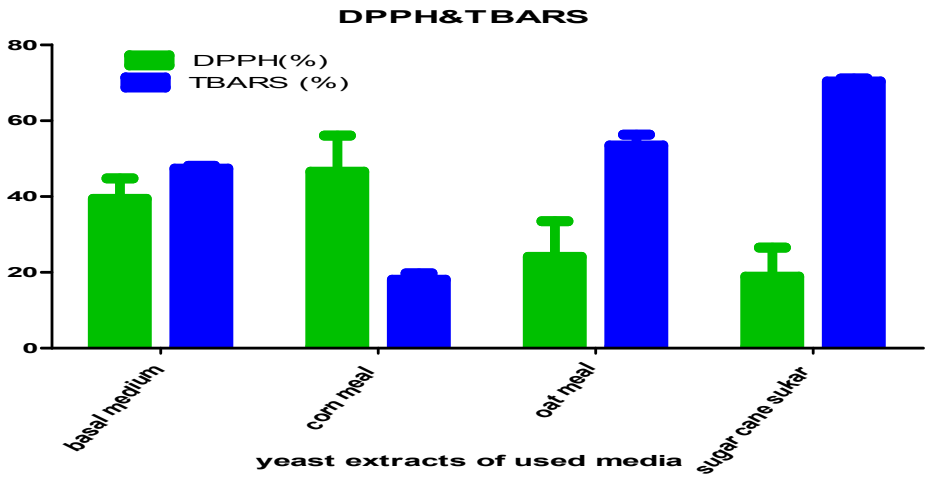



**Figure 1**: DPPH% and TBARS% of yeast extracts of four used media.

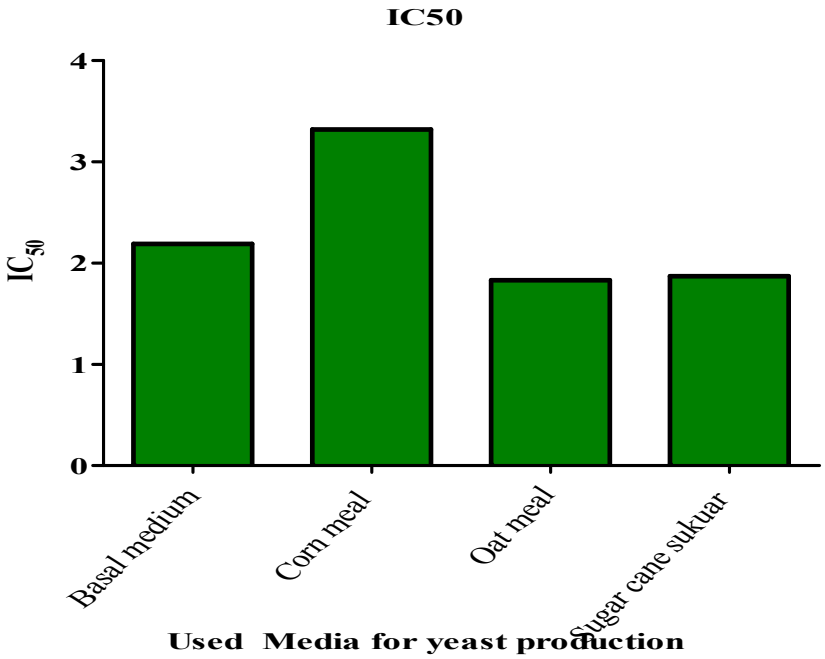



**Figure 2**: $IC_{50}$ values of yeast extracts produced by four used media.

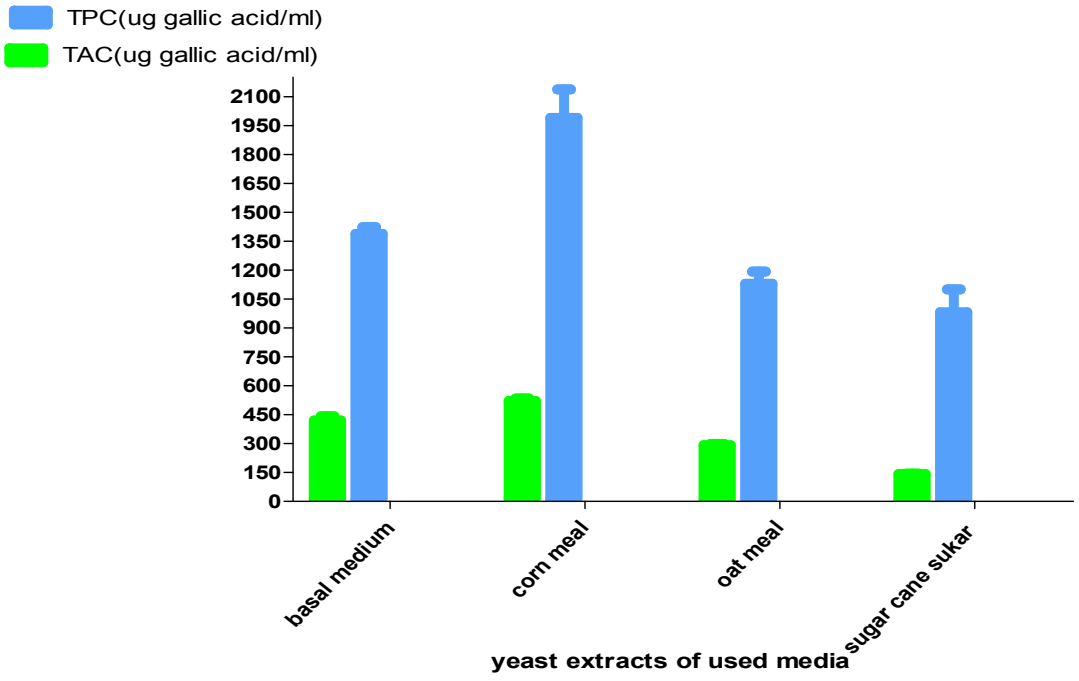



**Figure 3**: Total phenolic content (TPC) and Total antioxidant content (TAC) of yeast extract of four used media.

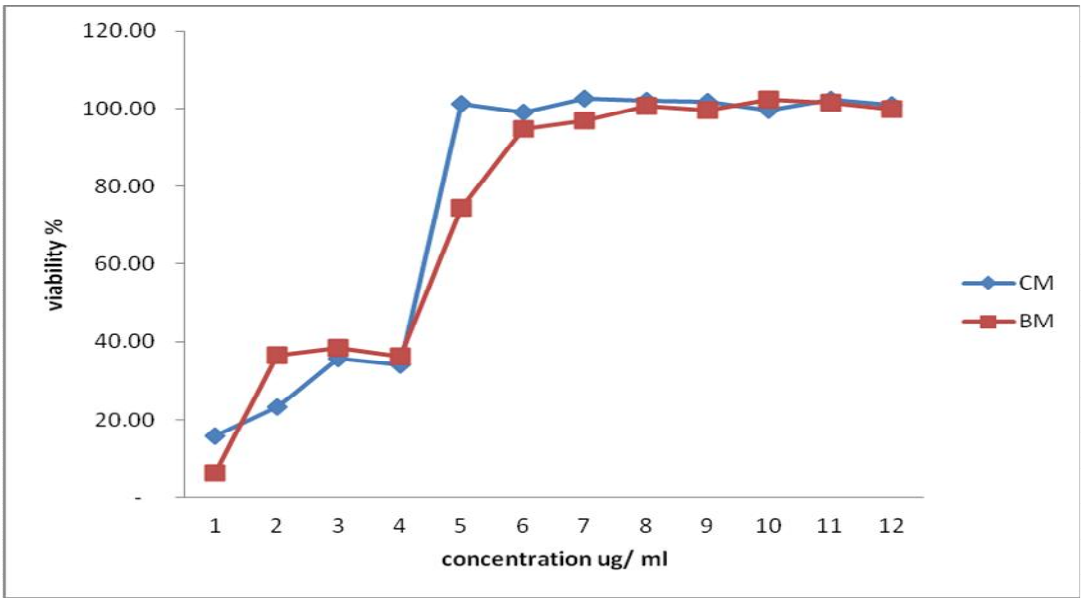



**Figure 4**: cytotoxicity by MTT assay for basal medium (BM) and corn meal (CM) on HepG2 cell lines.

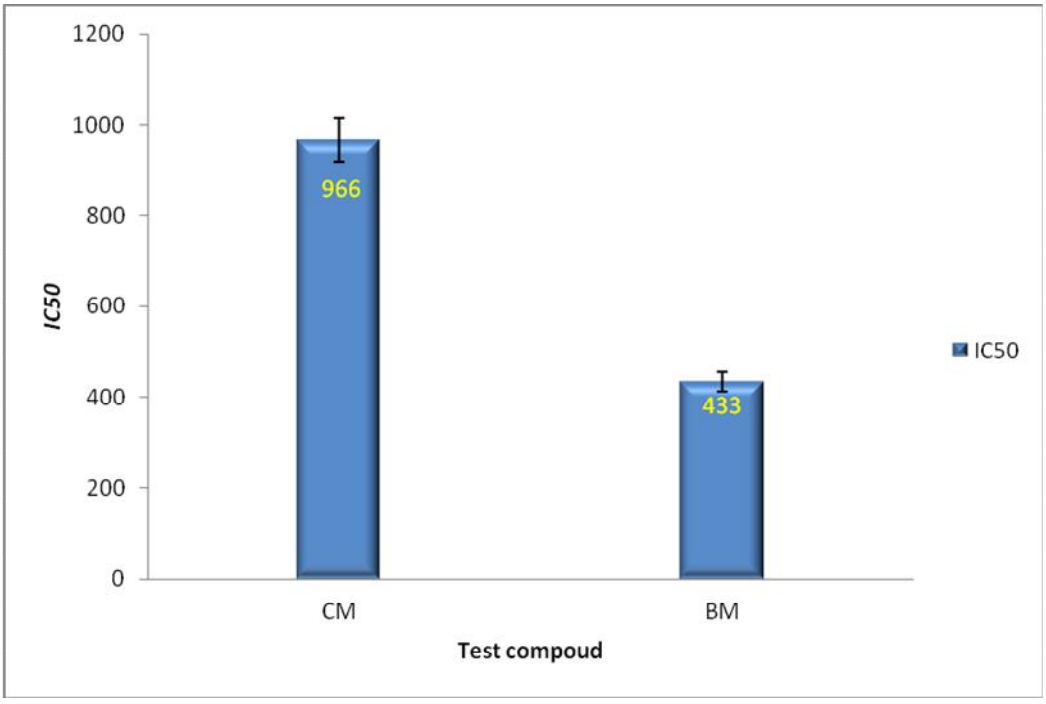



**Figure 5**: $IC_{50}$ for basal medium (BM) and corn meal (CM) on HepG2 cell lines.

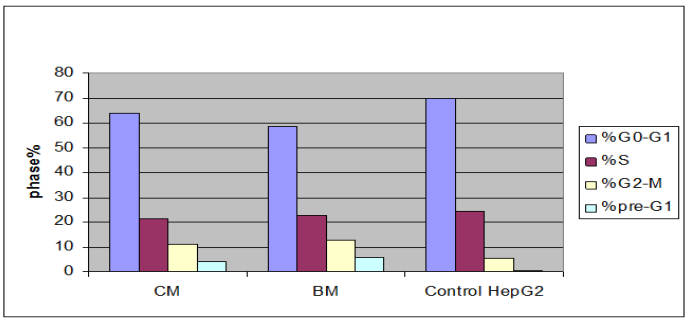
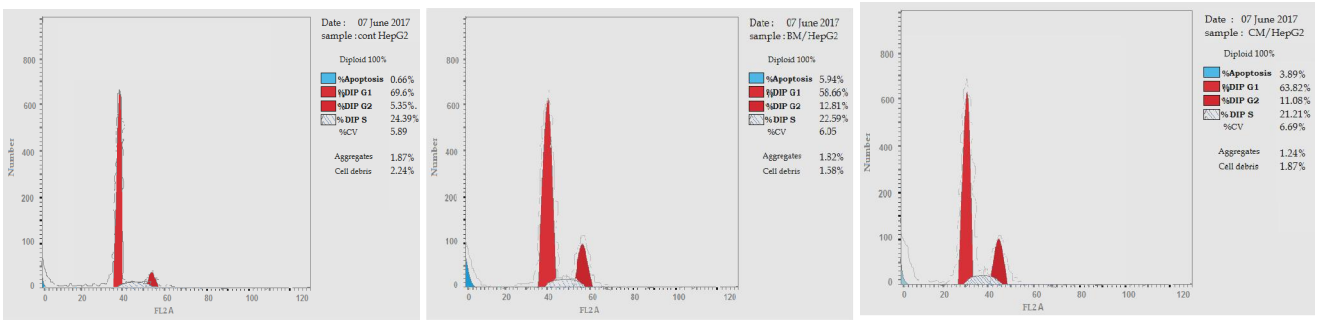
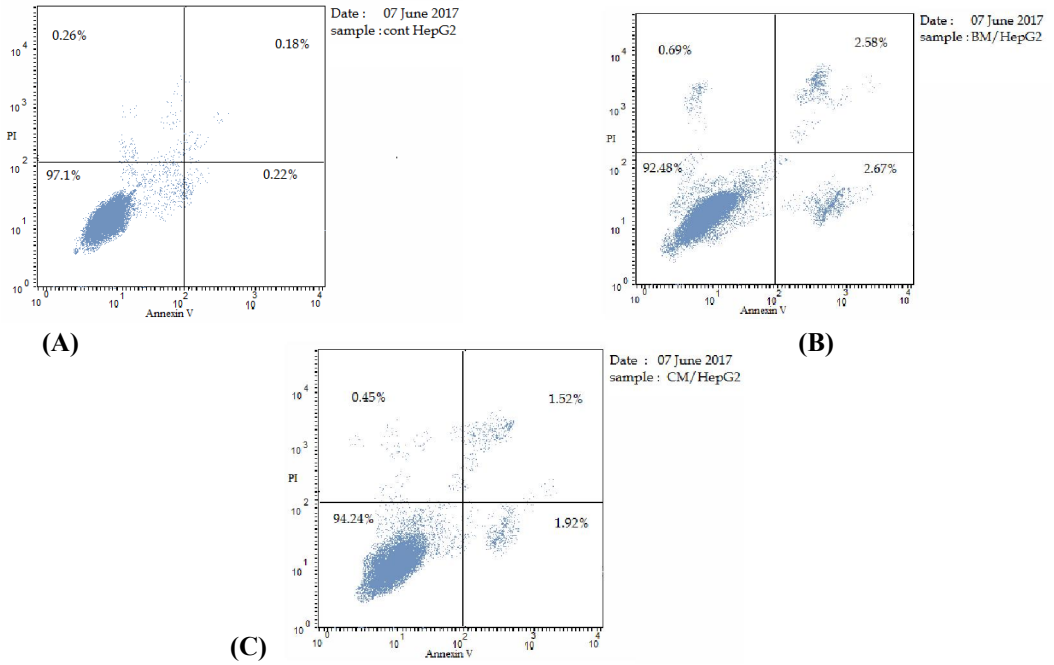



**Figure 6**: Phase (%) of two comparable yeast extracts (BM &CM) and control in HepG2 cell lines.




**(A)** **(B)** **(C)**

**Figure 7**: Cell cycle analysis of HepG2 cell line after treatment with BM(B) and CM(C) in comparison to control samples(A).




**(A)** **(B)** **(C)**

**Figure 8**: Flow cytometric analysis showing cell cycle arrest at G2/M phase and induction apoptosis after treating HepG2 cell line with basal medium (B) and corn meal (C) in comparison to control samples (A).

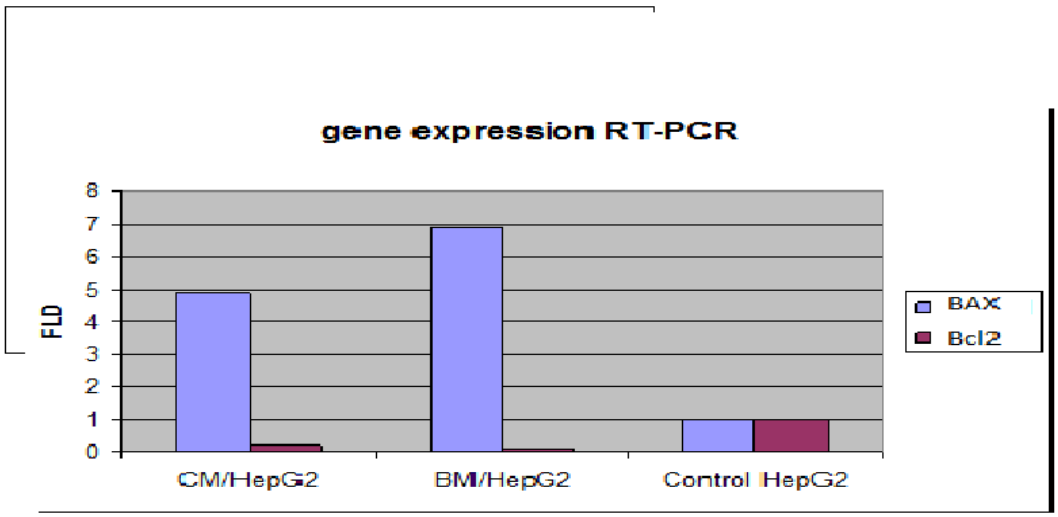



**Figure 9**: Expression levels of BAX and BCL2 genes in HepG2 cell lines after the treatment with BM and CM in comparison with control.

## DISCUSSION

The above results indicated that yeast fermentation had close relationship with differences in individual polyphenolic compounds, which is consistent with previous studies conducted by Loira et al. (2013) and Yanlai et al. (2011). This causes differences in physical and chemical reactions and leads to different structures and concentrations of phenolic compounds in the media. Undoubtedly, the choice of yeast strain dramatically influences phenolic composition and contents

It is well known that the proton-radical scavenging action is an important mechanism of antioxidant. Because DPPH possesses a proton free radical and shows a characteristic absorption at 517 nm, when it encountered proton-radical scavengers and the purple color of DPPH solution will fade rapidly. DPPH is usually used to determine the proton-radical scavenging action of the antioxidant. The obtained results was agreed with Choi et al. (2000) and Sokmen et al. (2004).

$IC_{50}$ of the represented work was decreased in case of basal medium while increased in case of corn meal. This data agreed with Meyer et al. (1982) and Ayo et al. (2007).

Phenolic substances and flavonoids are associated with antioxidant activity and play important role in stabilizing lipid peroxidation (Cross et al., 1987; Liu et al., 2012; Joyce, 1987; Liu et al., 2012) by adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng and Wang, 2001; Dorman et al., 2003)

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing a number of carbon-carbon double bonds. A large number toxic by-product is formed during lipid peroxidation. These have effect at site away formed area of their generation. Hence, they behave as toxic second messengers' (Devasagayam et al., 2003).

The yeast extract of corn meal gave better anticancer activity than basal medium this is due to Phenolic compounds, which are important secondary metabolites; possess various biological activities, the most important of which is antioxidant activity

associated with reduced cancer risk (Manach et al., 2005; Picchi et al., 2012).

## Conclusion

The outcomes of our study along with the conclusions and results of previous researches that were carried out to test the antioxidant, anti-cancer potential of, yeast extracts on natural commercial media more than synthetic media has a great potential as a cancer therapy.

## ACKNOWLEDGMENTS

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