Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2015; 7(6); 287-296

ISSN: 0975-5160

Research Article

Antioxidant and Anticancer Agents Produced from Pineapple Waste by Solid State Fermentation

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Available Online:29th November, 2016

ABSTRACT

Natural products are economically beneficial, safe and had promising effect. The natural sources such as plants, fruits and vegetables are rich in bioactive compounds which are valuable products for pharmaceutical industry. In fruit processing industry, large volumes of its residual were dumped and thrown as waste material which will be useful if it can be exploited for some beneficial purpose. So the aim of this study was to investigate the ability of *Kluyveromyces marxianus* NRRL Y-8281 to produce valuable products from pineapple waste. The phenolic content of methanolic extracts of unfermented (UFPW) and fermented (FPW) pineapple waste reached 112 and 120 mg Gallic acid/100 g dry waste respectively at concentration of 8 mg/ml. The antioxidant behavior of both methanolic extracts was determined by different methods. The highest levels of antioxidant activities were achieved with FPW extract. The *in vitro* anticancer activity of both extracts has been assessed against different human cancer cell lines. The results revealed that although both extracts did not show any effect against HepG2, HL-60 or normal HFB4 cells, they exerted anticancer effect closed to the value of the doxorubicin drug against MCF-7, A549 and HCT116 cell lines. The fermented extract was more potent than the unfermented one. GC/MS analysis was carried out to find out the nature of the compounds responsible for the antioxidant and anticancer activities. From forgoing results, the extracts of pineapple wastes as such or fermented one can be used as a good candidate for novel therapeutic strategies for cancer.

Keywords: Solid state fermentation, *Kluyveromyces marxianus*, pineapple waste, antioxidant, anticancer, GC/MS analysis.

INTRODUCTION

Waste disposal represents a serious problem to many agro industries since it is usually prone to microbial spoilage and causes major environmental problems. The utilization of agro-industrial waste by conversion into value added products may be an innovative solution to the environmental waste problem. Agro-industrial wastes in recent times have been the focus of research in drug design and in treatment a range of ailments[1]. Pineapple is the common name of *Ananas comosus* (*Syns. A. sativus, Ananassa sativa, Bromelia ananas, B. comosa*). Pineapple is the leading edible member of the family Bromeliaceae, grown in several tropical and subtropical countries including Philippines, Thailand, Indonesia, Malaysia, Kenya, India, China and South America. It has been used as a medicinal plant in several native cultures[2] and these medicinal qualities of pineapple are attributed to bromelain (EC 3.4.22.32), which is a crude extract from pineapple that contains, among other compounds, various closely related proteinases, exhibiting various fibrinolytic, antiedematous, antithrombotic, and anti-inflammatory activities *in vitro* and *in vivo*. When the pineapple fruits are canned or consumed the crown, the outer peel and the central core are discarded as pineapple waste which accounts for about 50% of the total pineapple fruit weight corresponding to about ten tons of fresh pineapple or one ton of dry pineapple waste per hectare[3]. Pineapple wastes are recommended as tremendous sources of organic raw materials and are potentially available for conversion into useful products[1,2,4]. It contains high amounts of crude fiber and suitable sugars for growth of microorganisms[5]. Moreover, Lateef *et al*.[6]; Rashad *et al*.[7,8] mentioned that fermentation process is necessary to improve the nutritional value of agro-industrial wastes, thereby offering the potential to make dramatic contributions to sustainable livestock production has been well documented. Solid-state bioprocessing consists of the utilization of water-insoluble substrates for microbial growth and it is usually carried out in solid or semi-solid systems in the near absence of water[9]. Biological conversion of food processing wastes have been successfully converted into many value-added products through solid-state bioprocessing[10-13]. The fruit and vegetable solid wastes

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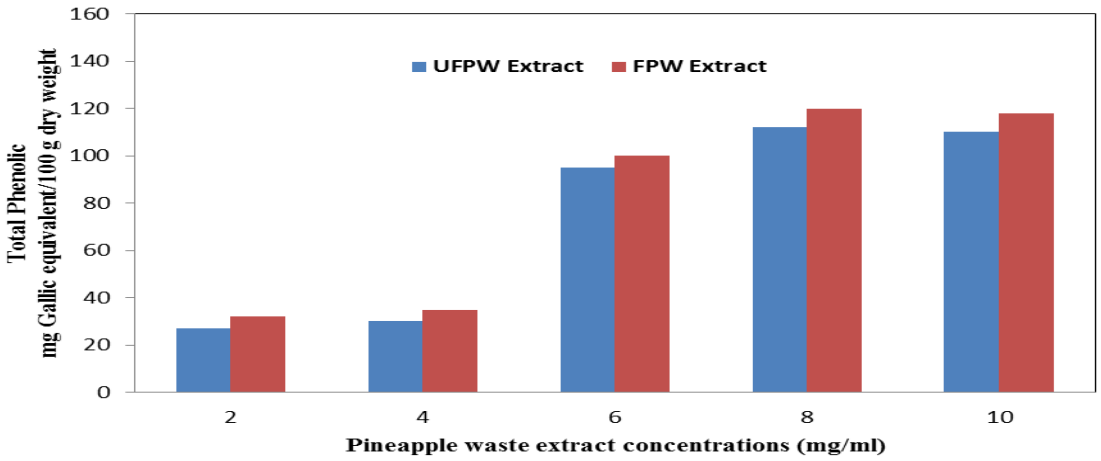

Figure 1: Total phenolic content of unfermented (UFPW) and fermented (FPW) pineapple waste extracts at different concentrations.

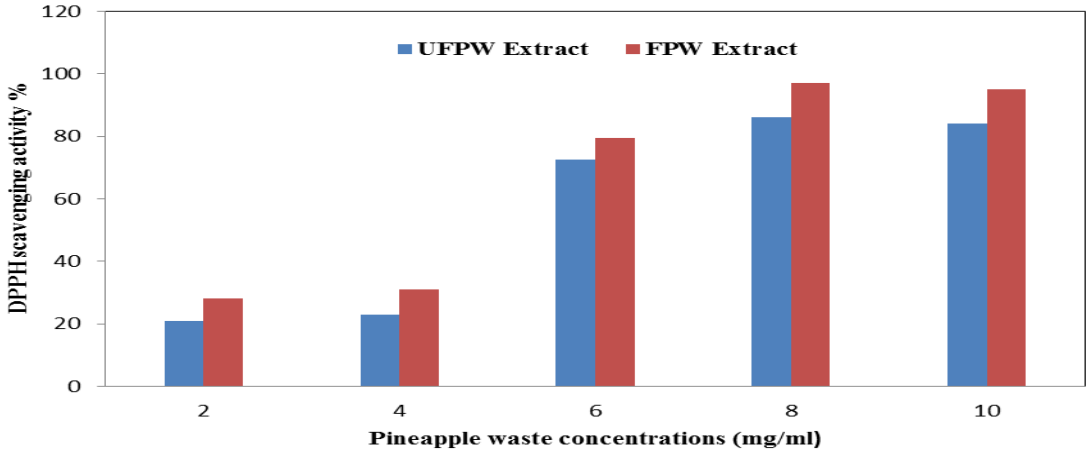

Figure 2: DPPH free radical scavenging effects of different concentrations of unfermented (UFPW) and fermented (FPW) pineapple waste extracts.

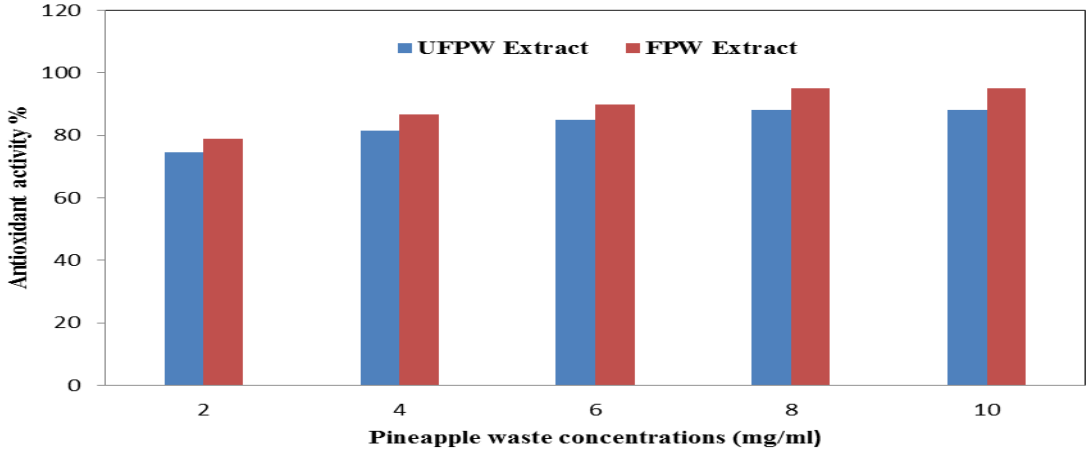

Figure 3: Antioxidant activity of different concentrations of unfermented (UFPW) and fermented (FPW) pineapple waste extracts in the β-carotene / linoleic acid system assay.

contain mainly soluble sugar and other hydrolysable materials and fibre. Disposal of such wastes may present an added cost to processors and direct disposal to soil or landfill may cause serious environmental problems. Thus, investigation and development of potential value-added processes for these wastes can be highly attractive[14].

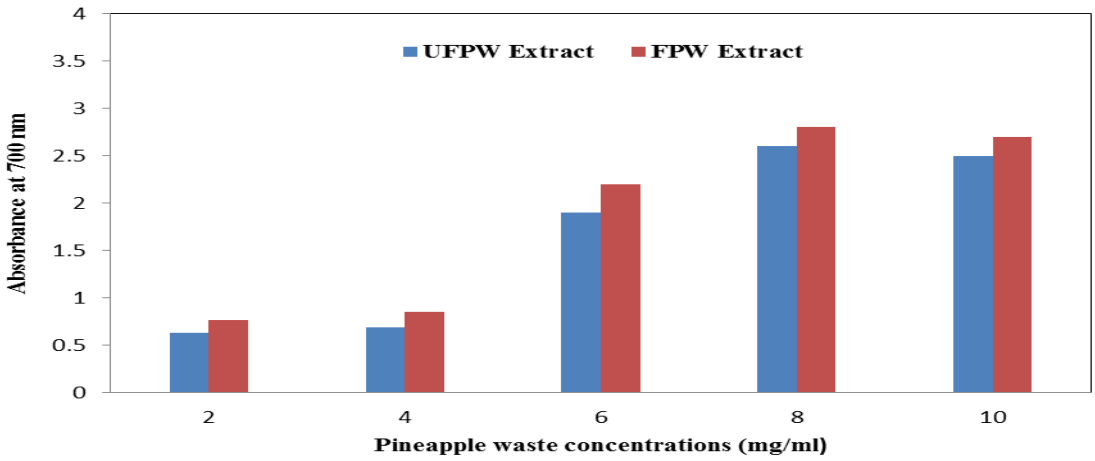

Figure 4: Reducing power of different concentrations of unfermented (UFPW) and fermented (FPW) pineapple waste extracts.

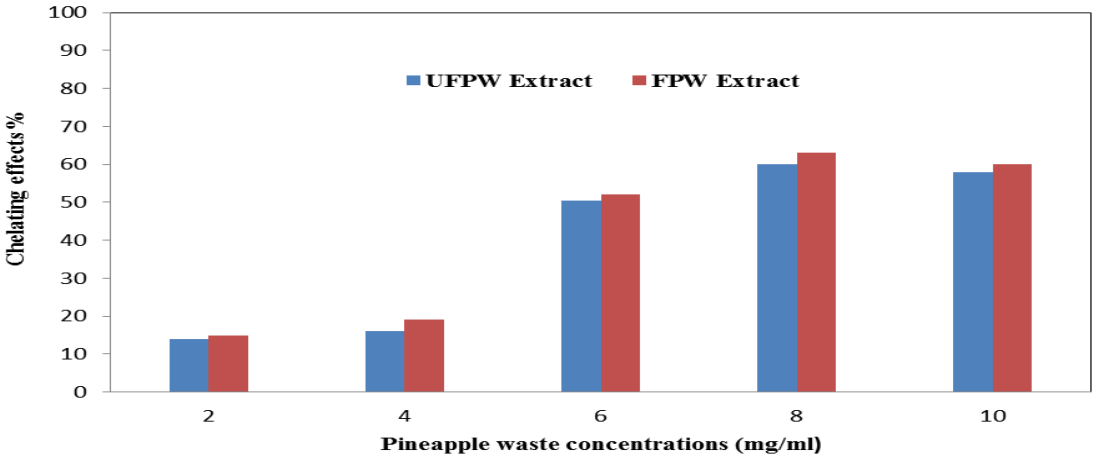

Figure 5: Chelating ability of different concentrations of unfermented (UFPW) and fermented (FPW) pineapple waste extracts.

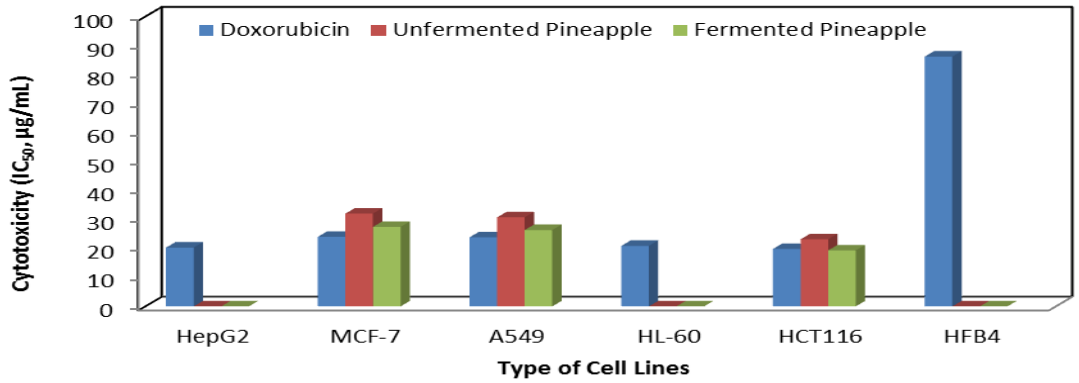

Figure 6: The *In vitro* anticancer activity of the tested extracts in different human cell lines as measured with SRB assay. The data were expressed as mean of four separate experiments.

Lately it has been reported that reactive oxygen species (ROS) are implicated in a large number of human diseases. When an imbalance between antioxidants and generation of ROS occurs, oxidative damage can occur and generate a large number of health problems such as arteriosclerosis and cancer[15]. Therefore, there is increased interest focused on natural antioxidants present in foods and medicinal plants because of their safety over the synthetic ones[16]. The present study was undertaken to test the feasibility of pineapple residue as a substrate for production of phenolic compounds have antioxidant activity by *Kluyveromyces marxianus* NRRL Y-8281 via

Table 1: Approximate composition (g %) of unfermented (UFPW) and fermented (FPW) pineapple waste.

Component	*UFPW*	*FPW*
Moisture content	68.00	69.00
Protein	5.06	6.00
Carbohydrate	56.20	56.60
Crude fat	3.00	3.44
Crude fibre	2.33	5.50
Ash	3.40	2.70

Values are expressed on dry weight basis.

SSF. Also, the change in the chemical composition of the waste during fermentation was investigated. Furthermore, the study was extended to highlight the potent anticancer activity of the methanolic extracts of unfermented and fermented pineapple waste against different human cancer cell lines including liver HepG2, breast MCF-7, lung A549, acute myeloid leukemia HL-60 and colon HCT116. Phytochemical profiling through GC/MS of these extracts were taken up so as to get insight into the compounds responsible for the antioxidant and anticancer activities and thereby pave way for indirect waste management by transforming these wastes into a health resource.

MATERIALS AND METHODS

Substrate

Pineapple waste is a by-product of the pineapple processing industry and consists basically of the residual pulp, peels, skin, core and crown. It was generously provided from Juice extraction shops located in Cairo, Egypt. It was collected freshly, washed with tap water, sliced, crushed in a mixer and store in freezer till used.

Microorganism

Kluyveromyces marxianus NRRL Y-8281 was obtained from Agricultural Research Service, Peoria, Illinois, USA. The strain was maintained on yeast malt agar[17], then stored at 4 °C and sub-cultured monthly. Inoculum was developed by transferring a loopful of stock culture into a sterile yeast malt medium[17] and incubated at 30 °C on a shaker at 200 rpm for 24 h.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Chemika, Folin–Ciocalteu reagent, gallic acid, ferrozine, β-carotene and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Solid state fermentation of pineapple waste

Fifty gram fresh pineapple waste was placed in 250 ml Erlenmeyer flasks and autoclaved at 121 °C for 15 min, cooled and then sprayed with 1.0 ml of *K. marxianus* NRRL Y-8281 (10^8/ml) inoculum. After mixing thoroughly, the inoculated pineapple waste was then incubated statically for three days at 30 °C. During the cultivation period, the culture was periodically stirred under sterilized conditions to accelerate the release of fermentation heat[8,12,18]. The unfermented pineapple waste used as control was prepared without the addition of inoculum. The unfermented pineapple waste (UFPW) and fermented one (FPW) were collected, dried at 60 °C, ground and then used for analysis.

Nutritional composition

Ash, fat and crude fiber contents of both fermented and unfermented pineapple wastes were determined following the methods of A.O.A.C.[19], while the crude protein contents were determined using micro-Kjeldahl method (N x 6.25). The carbohydrate content was determined by Dubois *et al*.[20] method.

Methanolic extracts

The fermented and unfermented pineapple wastes were extracted with methanol (1:5 w/v) at 55 °C for 2 h in a shaking water bath at 100 rpm. After filtering through Whatman No. 1 filter paper, the extracts were concentrated under vacuum at 30 °C[6].

Total phenolics content

Total phenolics content of the extract were estimated as gallic acid equivalents essentially according to that described by Quettier-Deleu *et al*.[21] with minor modification. An aliquot of 0.5 ml methanol extract was added to 7.0 ml deionized water and 5.0 ml Folin-Ciocalteu phenol reagent. After 3 min, 2.0 ml of 20% Na_2CO_3 were added and heated in a boiling water bath for 1 min comparatively to gallic acid standard. Absorbance was measured at 750 nm after cooling in darkness and the results expressed in mg of gallic acid/g dry substrate.

Antioxidant activities

DPPH radical-scavenging assay

The modified methods of Shimada *et al*.[22]; Mensor *et al*.[23] were used to study the free radical-scavenging activities of the extracts using DPPH. Two ml of methanolic extracts at various concentrations (2-10 mg/ml) were added to 1.0 ml methanolic solution of 0.3 mM DPPH. The mixture was shaken and left in a dark box to stand for 30 min at room temperature (30 ± 1 °C). The blank of each sample was prepared with 2.0 ml of sample solution with 1.0 ml of methanol instead of DPPH, while 1.0 ml of methanolic DPPH plus 2.0 ml of methanol served as control. The absorbance of the resulting solution was measured at 517 nm. The inhibitory percentage of DPPH was calculated according to the following equation:

Scavenging activity (%) = [1 - (absorbance sample/absorbance control)] x 100%.

β-carotene /linoleic system assay

Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was evaluated by a modification of the method described by Juntachote and Berghofer[24]. The β-carotene (2 mg) was dissolved in 20 ml of chloroform. An aliquot (3 ml) of the solution was

Table 2: Compounds identified of the major retention peaks obtained by GC/MS analysis in the methanolic extract of UFPW.

Compound	*R. T. (min)*	*M. Wt.*	*Area (%)*
Trimethylsilylmethanol	8.16	104	2.71
2,4-Decadienal,(E,E)-(CAS)	25.91	152	2.92
(E)-2-(2H(1)-4-Methoxyphenylethene	26.24	134	2.96
Pulegone	26.68	152	6.23
1-(4-Methoxyphenyl)-2-pentene-1,4-dione-4dimethylhydrazone	33.03	246	27.93
Hexadecanoic acid(CAS)	44.92	256	3.26
Di-2-benzothiazole disulfane	49.05	332	2.24
Fumaric acid, decyl2-dimethylaminoethylester	52.16	327	2.45
7-phenyl-10,10-dimethyl-8,9,10,11-tetrahydrobenz[c]acridin-8 one	55.66	351	3.26
Ç-Sitosterol	61.23	414	11.09
Stigmast-5-en-3-ol, (3á,24s)			
Pregn-5-en-20-one,3,17-dihydroxy-,(3á)-(CAS)	62.17	332	7.73
Lup-20(29)-en-3-one	63.80	424	2.77

R.T.: Retention time, M. Wt: Molecular weight

Table 3: Compounds identified of the major retention peaks obtained by GC/MS analysis in the methanolic extract of FPW.

Compounds	*R. T. (min)*	*M. Wt.*	*Area (%)*
Ni(ii)-2,7-bis[2-hydroxy-5,5-dimethyl-4,5-dihydro-1H-pyrrol-4-on-3-yl]-3,6-diazaocta-2,6-dien	5.59	418	3.63
2-Methyl-5-trideuteromethyltetrazole	8.18	98	4.71
Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]	11.51	442	3.09
2,4-Decadienal	25.91	152	3.52
2-hexylfuran	26.68	152	9.60
7,8-dimethoxy-2,2-dimethyl-2H-chromene	33.03	220	48.55

R.T.: Retention time, M. Wt: Molecular weight

mixed with 40 mg linoleic acid and 400 mg Tween 20 in a 150 ml beaker. Chloroform was removed and then 100 ml of oxygenated deionized water was added into the β-carotene emulsion and vigorously mixed until completely homogenized. An aliquot (3 ml) of this β-carotene emulsion and 0.1 ml of sample were placed in a capped culture tube and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50°C. Absorbance was measured at 470 nm at interval time (0, 10, 20, 30, 40 min). A control was prepared by using 0.1 ml of distilled water instead of the sample. Degradation rate of the sample was calculated according to the first order kinetics using Eq. (1): Ln (a/b) x 1/*t* = sample degradation rate; where Ln, natural log; a, initial absorbance at time zero; b, absorbance at time 40 min (time of discoloration); t, time (min). The antioxidant activity (AA) was expressed as % inhibition relative to the control using Eq. (2) AA (%) = 100 x (Degradation rate of control - Degradation rate of sample)/Degradation rate of control.

Reducing power

The reducing activity of the samples was determined essentially following the method of Oyaizu [25]. An equal volume (0.3 ml) of sample, potassium ferricyanide 1.0% and sodium phosphate buffer (0.20 M, pH6.6) were mixed thoroughly. The mixture was incubated at 50 °C for 20 min and then 0.3 ml of 10% trichloroacetic acid was added. The mixture was centrifuged (6000 rpm) at 4 °C for 10 min. The upper layer (0.6 ml) was mixed gently with 0.12 ml of 0.1% ferric chloride and deionized water (0.6 ml). After 10 min of mixing, absorbance of this mixture was measured at 700 nm. A higher absorbance of this mixture indicates a higher reducing activity.

Chelating effects on ferrous ions

Fe^{2+}-chelating ability of each extract was determined according to the method of Decker and Welch[26]. The Fe^{2+} level was monitored by measuring the formation of the ferrous ion–ferrozine complex. One milliliter of each methanolic extract was mixed with 3.7 ml methanol, 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine and the mixture was shaken and left at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm. A lower absorbance indicates a stronger Fe^{2+}-chelating ability. The ability to chelate the ferrous ion was calculated as follows:

Chelating effect (%) = [1- absorbance $_{sample}$ / absorbance $_{control}$] x100%.

Cell lines and culturing

Anticancer activity screening for the tested compounds utilizing liver HepG2, breast MCF-7, lung A549, acute myeloid leukemia HL-60 and colon HCT116 cancer cell lines as well as the normal cell line (human normal

melanocyte, HFB4) were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 ºC in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of culture medium.

In Vitro anticancer assay

The cytotoxicity activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure[27]. Cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. The tested compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds and doxorubicin were added to the cells. Triplicate wells were prepared for each individual dose. Cells were incubated with the compounds for 48 h. at 37°C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured at 492 nm in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in fig 6. The results were compared to the antiproliferative effects of the reference control doxorubicin[28].

Gas chromatographic-mass spectrometric (GC/MS) analysis

About 5 µl of each sample was used. The analysis was performed by using Thermo Scientific trace GC Ultra Couple with single quadrupole MS. A fused silica capillary column TG-5MS (30 m x 0.251 mm, 0.1 mm film thickness) was used. The oven temperature was maintained initially at 40°C for 3 min., and then programmed from 40 to 280°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate of 1 ml/min. The quantification of all the identified compounds was investigated using a percent relative peak area. A tentative identification of the components was performed based on their relative retention time and mass spectra with those of National Institute of Standard and Technology, NIST Willy library data of GC/MS system.

RESULTS AND DISCUSSION

Since fruit waste is rich in carbohydrate and other nutrients, it can serve as a substrate for production of useful metabolites via SSF. In this study the changes of chemical composition of pineapple waste was evaluated during their fermentation (Table 1). Major changes in the chemical composition were registered for protein, crude fiber and ash content. The protein mass fraction and crude fiber increased 1.2 and 2.5 times, respectively after fermentation, while in the same time the ash content decreased 20.5%. These values are in the range with the amounts present in fiber concentrates obtained from pineapple co-products (4% protein, 4.5% ash, 1.3% fat and 14.4% carbohydrates) as reported by Martìnez *et al*.[4]. So, the pineapple waste is not considered attractive as an animal feed, since it contains, on a dry matter basis, high fibre content and soluble carbohydrates, as well as low protein content[29].

Extraction efficiency

The antioxidant activity of ethanol, toluene, petroleum ether, acetone, methanol and water extracts of pineapple peels and co-products have been reported in the various *in vitro* models[4,16]. The methanol extract exhibited the highest activity and extraction yield. Hence, in the present study the methanolic extract of pineapple waste has been used. The methanolic extraction yield of UFPW and FPW was (57%) which is higher than the results of Gnanasaraswathi *et al*.[16] who reported that the yield of extract of pineapple peels was 11.8 %.

Total phenolic content

Polyphenolic compounds are very important fruit constituents due to their antioxidant activities, their chelation of redox-active metal ions, inactivation of lipid free radical chains and prevention of hydroperoxide conversion into reactive oxyradicals[30]. Phenolic content can be used as an important indicator of antioxidant capacity and can be used as a preliminary screen for any product when intended to be used as a natural source of antioxidants in functional foods[31]. The total phenolic content of the methanol extract of FPW with various concentrations (2-10 mg/ml) was shown in Fig (1). All were higher than that of UFPW extract. FPW extract exhibited the highest phenolic content (120 mg GAE /100 g dry weight) at concentration of 8 mg/ml, also at the same concentration the UFWP one exhibited its highest phenolic content (112 mg GAE /g dry weight). The total phenolic contents of the two extracts decreased gradually with further increasing in the concentration (Fig 1). These values are within the broad range reported in the literature for co-products and peels of exotic fruit such as pineapple (129 mg GAE/100 g), mango (283 mg GAE/100 g), guava (39 mg GAE/100 g)[4], banana passion fruit (246 mg GAE/ 100 g), cocona (87.4 mg GAE/100 g), cupuaçu (252 mg GAE/ 100 g)[32]. While our results are less than that reported for whole fruit such as zapote (258 mg GAE/100 g), cherimoya (323 mg GAE/100 g), or strawberry (238 mg GAE/100 g),[33]. The concentration and type of phenolic substances in fruit and fruit co-products depend on several factors; differences in varieties, ripeness and season; environmental factors, such as soil type and climate; genetic factors and processing and extraction methods. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity plays a key role in increasing phenolic solubility[34].

Antioxidant activities

Fruits contain many compounds that display antioxidant functionalities. Therefore, to measure the antioxidant capacity of each compound individually is a complex and difficult undertaking. Several methods have been developed to estimate the total antioxidant capacity of different plant materials[35]. Usually, these methods measure the ability of antioxidants to scavenge specific radicals, to inhibit lipid peroxidation or to chelate metal ions. The use of simple "total antioxidant capacity'' methods differing in their way of generating free radicals, the strategy to measure the end point of the inhibition reaction, and the sensitivity towards the different reducing molecules in the sample[36]. Therefore, more than one method should be used to gain a more complete picture of the antioxidant capacity of phenolic compounds obtained from the methanolic extracts of unfermented and fermented pineapple waste.

DPPH radical scavenging activity

The DPPH method measures the ability of certain extract phytochemicals to scavenge free radicals and in doing so, it is a useful analysis to understand whether the antioxidant enriched extract can block the oxidation initiation phase by the ability to neutralize or inhibit the formation of radical species[37]. From Fig. 2, a gradual increase in the scavenging activity was observed which reached a maximum of 86% for UFPW extract and 97% for FPW extract at the concentration of 8 mg/ml and beyond this concentration a decrease in their activities was observed. Obviously, there were more antioxidant components present in FPW than in UFPW, which could react rapidly with DPPH radicals, and reduce almost all DPPH radical molecules corresponding to available hydroxyl groups[38]. This result reveals that both extracts potently scavenged DPPH radicals. The ability of extracts to scavenge these radicals suggests that it contains compounds that are electron donors, which can react with free radicals to convert them to more stable products and terminate radical chain reaction. Thus, DPPH scavenging may be related to inhibition of lipid peroxidation. Scavenging of DPPH radicals has also been reported for fermented pineapple waste mixed with soy flower extract[39] and pineapple co-product extract[4]. DPPH scavenging activity of methanolic extracts of UFPW and FPW obtained in this study is higher than that of methanolic extracts of peels (66.6%) as obtained by Gnanasaraswathi *et al*.[16].

β-Carotene / linoleic acid system assay

The β-carotene assay quantifies the ability of the antioxidant to act at a lipid–water interface. The antioxidant protection factor directly quantifies the capacity of the antioxidant enriched extract to prevent the oxidation of β-carotene catalyzed by the presence of H_2O_2 [10]. The β-carotene oxidation assay was evaluated for both UFPW and FPW extracts (Fig. 3). It was observed that, both extracts exhibited an excellent antioxidant activities ranged from 74.5 to 95% at various concentrations (2-10 mg/ml). This indicated that both UFPW and FPW extracts exhibited the peroxidation of linoleic acid. In general the linoleic acid radical scavenging activity of pineapple waste was enhanced after fermentation. From the figure, it is clear that FPW extract showed a more potent capacity to suppress lipid peroxidation when compared with unfermented one and concentration dependent effects were observed. The percent of activities of UFPW and FPW extracts reached 88 and 95% respectively at 8 mg/ml concentration and remained constant thereafter.

Reducing activity

The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity. From the results (Fig. 4) it was found that the reducing power of both UFPW and FPW extracts increased with the increase in concentrations till 8 mg/ml and beyond this concentration a decrease in reducing power was observed. The better reducing power is obtained with the fermented substrate. This results shows that the pineapple waste extract and its fermented form possess reducing power capabilities and act as a potent antioxidant.

Ferrous ion chelating ability

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food[40]. The catalysis of metal ions also correlates with incidents of cancer and arthritis[41]. Ferrous ions, the most effective pro-oxidants, are commonly found in food systems[42]. In the present study, the chelating ability of the UFPW and FPW extracts toward ferrous ions was investigated. As shown in Fig. 5, the two methanolic extracts tested exhibited Fe^{2+}-ion chelating effect. At the dosage level of 8 mg/ml, both UFPW and FPW extracts, exhibited a maximum chelating effect of 60 and 62% respectively for Fe^{2+} ions. This indicated that both fermented and unfermented pineapple waste was good chelators for ferrous ions.

In vitro anticancer activity

As shown in Fig. 6, the antiproliferative activity of the unfermented and fermented pineapple extracts was tested using SRB assay in HepG2, MCF-7, A549, HL-60 and HCT116 cancer cell lines as well as human normal melanocyte, HFB4. For comparison, doxorubicin was also tested as positive drug. While treatment with DMSO was used as control cancer cells. Moreover, the tumor cells showed normal growth in culture system and DMSO did not seem to have any noticeable effect on cellular growth. Additionally, both extracts exhibited no activity against the growth of normal HFB4 cells as well as HepG2 and HL-60 cells.

From the results, it is evident that the extract of unfermented pineapple displayed potent anticancer activity against MCF-7, A549 and HCT116 with IC_{50} value of 32.00±3.50, 30.72±3.80 and 23.16±3.22 µg/ml respectively, near to the IC_{50} of doxorubicin (IC_{50}: 24.00±2.72, 23.84±2.43 and 19.83±2.11 µg/ml, respectively). on the other hand, the extract of the fermented pineapple displayed potent anticancer activity against MCF-7, A549 and HCT116 with IC_{50} value of 27.43±2.81, 26.36±2.90 and 19.33±2.11 µg/ml closed to the IC_{50} values of the reference drug, doxorubicin (IC_{50}: 24.00±2.72, 23.84±2.43 and 19.83±2.11 µg/ml, respectively).

It is clear that both extracts exert anticancer effect closed to the value of the doxorubicin drug against MCF-7, A549 and HCT116 cell lines with no noticeable toxicity on the normal cells, especially the fermented extract was more potent than the unfermented one.

Gas chromatographic-mass spectrometric (GC/MS) analysis

The methanolic extracts of UFPW and FPW were further subjected to GC-MS analysis so as to investigate the type of compounds responsible for the observed promising antioxidant and anticancer activities. As seen from Table 2, which gives the compounds of the major retention peaks obtained in GC/MS analysis of methanolic extract of the UFPW showed 1-(4-Methoxyphenyl)-2-pentene-1,4-dione-4-dimethylhydrazone (hydrazone derivatives), β-Sitosterol (cholesterol compound), phenolic compounds (mainly (E)-2-(2H(1)-4-Methoxyphenylethene, Di-2-benzothiazole disulfane and 7-phenyl-10,10-dimethyl-8,9,10,11-tetrahydrobenz[c]acridin-8-one) as the prominent retention peaks. It is clearly visible that the 1-(4-Methoxyphenyl)-2-pentene-1,4-dione-4-dimethylhydrazone peak retention area is a maxmium (27.93%) followed by β-Sitosterol (11.09%) and the other prominent retention peaks of phenolic compounds, seems to have a vital role to play in the observed antioxidant activity.

Hydrazones have been demonstrated to possess, antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular and antitumoral activities[43]. The bioactive substances found in plant food inlcude phytosterols. Plant sterols or phytosterols are structurally similar to cholesterol and exist in several forms in plants[44], including β-sitosterol, campesterol, stigmasterol and cycloartenol (Table 2)[45]. Of these, sitosterol is the most abundant phytosterol, followed by campesterol[45,46]. These compounds may affect many metabolic processes[47]. In addition to their cholesterol-lowering actions, mounting evidence suggests that phytosterols possess anti-cancer effects[48] against cancer of the lung[49], stomach[50], ovary[51] and estrogen-dependent human breast cancer[52]. It has been speculated that phytosterols inhibit the production of carcinogens, cancer-cell growth, invasion and metastasis, and promote apoptosis of cancerous cells[53]. These observations imply that phytosterols may be useful in prevention of both cardiovascular disease and cancer. GC/MS analysis of methanolic extract of FPW (Table 3) revealed that the chemical classes- chromene, furanone and heterocyclic compounds to have a key role in the observed antioxidant and anticancer activities in this extract. Chromene (7,8-dimethoxy-2,2-dimethyl-2H-chromene) is the major retention area peak (48.55%) in the FPW extract, it appears as an important structural component in various natural products and also possess useful biological properties. The potency of these clinically useful pharmacophore in treatment of cancer and inflammation and other activities encouraged the development of some more potent and significant compounds[54]. Furanone (2-hexylfuran, peak area 9.6%), which is present naturally in fruits as an aroma compound and as pheromones[55], furanone derivatives, recently attracted much attention due to their unique structures and varied pharmacological activities, which include antioxidative, protein tyrosine kinase inhibitory, anticancer, protein tyrosine phosphatase 1B inhibitory, antithrombotic, antimicrobial, anti-inflammatory[56].In view of the results obtained, pineapple residue has shown promising potentials to be exploited as alternative substrate for natural antioxidant and anticancer production by *Kluyveromyces marxianus* NRRL Y-8281 via SSF process. Although, further evaluation performed with the pure compounds is required for confirming the contribution of the identified bio-compounds to the antioxidant and anticancer activities. This study definitely opens up the scope for further utilization of these waste products for therapeutic and industrial purpose.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support by the National Research Centre, Egypt (Project no.10130105)

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