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ESTIMATION OF TOTAL PHENOLIC, FLAVONOIDAL CONTENTS, ANTIOXIDANT ACTIVITY AND GC-MS OF RAW AND STABILIZED DEFATTED RICE BRAN

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ABSTRACT

The by-product of the rice milling procedure, which entails turning brown rice into polished rice, is rice bran. It has a sizable number of useful bioactive substances. The present study to investigate the rice bran ethanolic extract for Antioxidant assay and GC-MS analysis of three treatments: Defatted Un-treated rice bran, Defatted dry-heating rice bran, and Defatted autoclaving rice bran. *In vitro*, three different antioxidant techniques (DPPH, FRAP and ABTS) were estimated, Our result revealed that the Defatted Dry-heating Rice bran sample extract exhibited maximum inhibition, followed by the Defatted Autoclaving Rice Bran extract. The lowest level showed in Defatted Un-Treated Rice Bran extract. The more efficient treatment of rice bran extract is Defatted Dry- heating Rice bran in the total phenolic, total flavonoid compounds, and antioxidant assay compared to the other treatments for the study. The presence of seven different phytochemical components **of dry heating Rice bran extract**, each of which has an additional biological action, was detected by GC-MS, including the, Lanosta-8, 24-dien-3-ol, (3á), Isochiapin B, ά-Sitosterol and Ethyl iso-allocholate **.**

Keywords: Defatted Un-Treated Rice Bran, Defatted Dry-heating Rice bran, and Defatted Autoclaving Rice Bran, GC-MS analysis, $IC_{50.}$

INTRODUCTION

The Gramineae family includes the plant known as rice, *Oryza sativa* (Anwar *et al.,* 2005). In the vast majority of emerging nations, it serves as the primary cereal food crop. Over 50% of the world's population consumes rice as their main source of carbs, while Asian countries produce 95% of the world's rice (Muthayya *et al.,* 2014). The majority of the rice bran produced by the rice polishing sector is used as animal feed (Anwar *et al.,* 2005). The amount of anthocyanin that is deposited influences the colour of the rice coat, and rice bran makes up around 60% of the total nutrients and 8% of the grain's weight. (Chaudhary, 2003). The majority of it is made up of the pericarp, aleurone, sub-aleurone, and germ layers (Sharif et al., 2014).

Rice bran is a naturally occurring source of vitamins, minerals, protein, fat, fibre, and vital unsaturated fatty acids. It also contains antioxidants such phenolics, tocopherols, tocotrienols, and oryzanol (Sohail et al., 2017). Oryzanol is the primary bioactive substance in rice bran. Owing to its benefits against inflammation (Debnath et al., 2013), cancer (Yasukawa et al., 1998), hypocholesterolemia (Revilla et al., 2009), anti-oxidants (Xu et al., 2001), inflammation (Jariwalla, 2001), and diabetes (Jariwalla, 2001, Ghatak et al., 2012). Rice bran is also attracting a lot of interest from the food, nutraceutical, and pharmaceutical industries because of its high nutritional content, low cost, easy availability, high bioactivity potential, and related health advantages (Sohail *et al.,* 2017).

In order to minimise ant nutrients and block lipase, which hydrolyzes triglycerides into glycerol and free fatty acids once the rice grains are milled, it is unfit for human food and needs to be stabilised quickly (Saunders *et al.,* 1985). Yet, to a lesser extent, peroxidase and lipoxygenase are also responsible for the rancidity of rice bran (Orthoefer *et al.,* 1996). The aim of the study is to know the effect of some thermal treatments such as drying, autoclaving on phenolic and flavonoids content and antioxidant activity of rice bran

MATERIALS AND METHODS

Fresh rice bran powder was obtained from a local rice mill in Mansoura to stop the generation of fatty acids made without rice bran from rising which collected in December 2022.

Stabilization of Rice Bran: Untreated Rice Bran (Un-RB): 0.5 kg Untreated Rice Bran served as the control (Zaghlol *et al.,* 2018).

- 0.5kg Rice Bran was prepared for dry-heating **(DH-RB)** by spreading it in thin layers onto open pans and baking them for 40 min at 15 °C (Irakli *et al*., 2020).

-For autoclaving, fresh rice bran (0.5 kg) was added to a 2,000 ml beaker and covered with two layers of gauze. It was then sterilised using an autoclave and a vertical steam steriliser (DGL-75B, country) at 121°C for 20 min (Yu *et al.,* 2020).

Proximate Analysis

Utilizing accepted techniques, the amounts of moisture, protein, ash, and

fat were measured. For example, the total carbohydrates were calculated by difference, the amounts of protein were calculated using the Kjeldahl method, the amounts of ash were measured using the dry ashing method, and the amounts of fat were measured using a Soxhlet device (AOAC, 2016). Rice bran that has been fully fatted was soaked in hexane for the duration of a single night at room temperature. After three days of switching the solvent, the resulting solution was filtered, and the solvent was extracted using a rotary evaporator (Kahlon *et al.*, 1992). After soaking in hexane for the full night at room temperature, rice bran was defatted. After three days of switching the solvent, the resulting solution was filtered, and the solvent was extracted using a rotary evaporator (Kahlon *et al.*, 1992).

Free fatty acids (FFAs): Using a common titration technique, the free fatty acid (FFA) concentration of rice bran samples was ascertained (AOCS, 1989).

Obtaining Samples:

Each 5 g of the sample was extracted for 3 h at room temperature using a magnetic stirrer and 50 mL of 70% ethanol. An additional three h were spent extracting the residue, twice passing it through Whatman *No*. 1 filter paper before pouring it into petri dishes. The extracts were left in the open until the solvent volatilized with a few minor changes (Arab *et al*., 2011).

Analysis of phytochemicals quantitatively

Determine the total phenolic and flavonoid contents via colorimetry analysis:

Using the Folin-Ciocalteu colorimetric method, the total phenolic content was determined and represented as gallic acid equivalents (mg Gallic acid/g of extract) (Haq *et al.,* 2012). Using aluminium chloride colorimetry, the total flavonoids concentration was determined and expressed as quercetin equivalents (mg of quercetin/g of extract) (Chang *et al.,* 2002; Mahrous et al., 2023)

DPPH-based measurement of antioxidant capacity

Using GraphPad Prism 5, the concentrations were converted to their logarithmic values, and the nonlinear inhibitor regression equation (log (inhibitor) normalised response - variable slope equation) was selected in order to get the IC_{50} value (Boly *et al.,* 2016). The ability of rice bran extract to neutralise the stable free radical 2, 2-diphenyl-1-picryl-hydrazyl-hydrate was evaluated using the procedure described by (Zhang *et al.,* 2013; Mahrous et al., 2023)

Measurement of antioxidant capacity using ferric reducing antioxidant power (FRAP)

Benzi *et al.* (1996).employed the FRAP experiment to determine the reduction power of rice bran extract with a few modest modifications

Using the ABTS method to evaluate antioxidant capacity

The free radical ABTS (2, 2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) was tested to see if rice bran extracts could neutralise it Rice bran ethanol extract can be distinguished using gas from defatted dry heating rice bran (Arnao *et al.,* 2001).

Chromatography-mass spectrometry (GC-MS).

The GC-MS analysis was carried out by the National Research Center (NRC), Dokki, Giza, using a thermal Scientific, trace GC Ultra / ISQ Single Quadruple MS, and TG-5MS fused silica capillary column (30 m, 0.25 mm, 0.1 mm film thickness). Using an electron ionisation system with ionisation energy of 70 eV and a constant flow rate of 1 ml / min, helium was employed as the carrier gas for GC-MS detection. The MS transfers line and injector had a set 280^{o}C temperature. The quantification of all the identified components was evaluated using % relative-peak area. Based on a comparison of the respective retention times of the compounds, the mass spectra and data from the WILLY and NIST libraries of the GC-MS system were used to make a preliminary identification of the compounds.

Statistical evaluation

The averages and standard deviations of three parallel measurements made up the experimental data. The analysis of variance was carried out using ANOVA methods. GraphPad Software, San Diego, California, USA, developed GraphPad Prism to calculate statistics at $P< 0.05$ (Motulsky, 1999).

RESULTS AND DISCUSSION :

Chemical composition of raw-defatted and stabilized rice bran:

The moisture content of the samples ranged from 2.53% to 11.38%, with dry heating producing the lowest moisture content and defatted autoclaving rice bran (De -A -RB) having the highest. Table 1 lists the average values for the chemical composition of rice bran. According to Thanonkaew *et al.* (2012), moisture content varies with processing, temperature, and time.

Protein content of all samples is (10.64: 17.32 %)the highest value was (De –A –RB) , the lowest value 10.64% was for Untreated rice bran (Un-RB) This result in the same line with (Zaghlol *et al.,* 2018 ; El-Gammal *et al .,* 2017). Fat percentage from (23.61 % for Autoclaving rice bran (A-RB) to .09 % for Defatted Un Treated rice bran (De-Un-RB). The ash content was high in dry heating rice bran before and after defatting (8.82 – 9.94 %) respectively .agree with (El-Gammal *et al.,* 2017). With regard to fibers the defatted dry heating rice bran (De-DH-RB) was the top content of fibers, while the (Un-RB) was the least . (Zaghlol *et al.,* 2018).

Rice bran samples' carbohydrate content ranged from (37.05 to 56.13 %) (De -Un-RB) having the highest carbohydrate level (56.13%) according to (Abd El Salam, 2017). and low value was in (A-RB) 37.05%. As previously

reported by other scientists (Lakkakula *et al.,* 2004), different stabilising techniques may limit enzymatic activity to a greater or lesser amount, leading to enhanced or decreased oil extraction.

Quantitative Analysis of Phytochemicals:

- Phenolic and flavonoid content overall:

Because they can create stable radical intermediates and transfer electrons or hydrogen, phenolic acids have a reputation for serving as antioxidants that prevent the oxidation of a variety of foods, including fatty acids and oils (Cuvelier *et al.,* 1992). According to this investigation, the total phenolic content of the defatted rice bran extract ranged from 38.8 to 40.5 mg/g as gallic acid equivalents (GAE). The defatted dry heating rice bran extract (40.5mg/g) contained the highest concentration of total phenolic acids. The results are in agreement with those published by (Zaghlol *et al.,* 2018; Pokkanta *et al.,* 2022), and (Anandito *et al.,* 2019), but not in agreement with those published by (Irakli *et al.,* 2020). The lowest value was found in the defatted, untreated rice bran extract (38.8 mg/g). The range of total flavonoids was 4.96 to 9.93 mg/g. According to Pokkanta *et al.* (2022), the defatted dry heating rice bran extract had the greatest total flavonoids (9.93 mg/g), whereas the defatted autoclaving rice bran extract had the least value.

- Activity of Antioxidants:

Using free radical estimation, the antioxidant (scavenging) activity of several extracts was measured (DPPH). When DPPH is exposed to an electron or a hydrogen radical, it changes from a stable free radical to a stable diamagnetic molecule (David *et al.,* 2004). Antioxidants' effect on the DPPH radical's ability to be reduced is measured by the decrease in its absorbance at 517 nm. Since radicals are scavenged by hydrogen donation when an association between antioxidant molecules and radicals forms, antioxidants are to blame for the drop in DPPH radical absorption. It can be visualised as a change from purple to yellow in colour. Hence, DPPH is frequently used as a substrate to assess the effectiveness of antioxidants (Edamatsu *et al.,* 1989). The investigated rice bran extracts all shown free radical scavenging activity in the current data (Table 3). (De -DH -RB) extract exhibits the strongest DPPH scavenging capabilities along with (Anandito *et al.,* 2019). Defatted Un Treated Rice Bran, Defatted dry- heating rice bran, and Defatted Autoclaving Rice Bran all had mean IC_{50} values of ethanolic extract of 37.16, 36.78, and 36.79, respectively. On the other hand, the ABTS radical scavenging activity of Defatted Un - Treated Rice Bran, Defatted Dry- heating Rice Bran, and Defatted Autoclaving Rice Bran was 189.85, 284.46, and 210.

Awika *et al.* (2003) asserted that the ABTS assay is superior to the DPPH assay because it is quicker, less expensive, and operational over a larger pH range. Both of these radicals are routinely used to assess antioxidant efficacy in vitro despite not being present in biological systems. The FRAP radical scavenging activities of (De-Un-RB), (De-DH-RB), and (De-A-RB) were 135.07, 195.07, and 124.89 **µM TE/mg** sample, respectively. The ability of the

free and bound phenolic compounds in rice bran to convert iron (III) to iron (II) was evaluated using FRAP analysis. The free phenolic FRAP antioxidant ability was enhanced by the stabilisation treatment, according to Ti *et al.* (2014) and Wanyo *et al.* (2014). Moreover, bound phenolics demonstrate a clear correlation between ferrous reducing activity and total phenolic compound.

Free fatty acids (FFA):

Unstabilized rice bran oil had 3.96% more free fatty acids (FFA) than stabilised rice bran. When heated by dry heating (2.8%) and autoclaving (3.35%), stabilised rice bran produces oil of greater quality than unstabilized rice bran (Hussain *et al.*, 2021). It was assumed that the FFA concentration had increased due to the rice bran's enzymatic lipase activity. Stabilization is required before extraction in order to inhibit lipase enzymes and reduce the amount of FFA in the produced oil (Amarasinghe *et al.*, 2009).

Identification of defatted dry heating Rice bran extracts using gas chromatography-mass spectrometry (GC-MS).

According to the findings in Table 4 and Fig. 1, Defatted dry- heating rice bran rice bran ethanol extract contained seven different compounds that were identified using gas chromatography-mass spectrometry (GC-MS). The main substances present in the defatted dry heated rice bran ethanol extract included Lanosta-8, 24-dien-3-ol, (3á), Isochiapin B, ά-Sitosterol , Ethyl iso-allocholate , 1-Heptatriacotcanol, 4H-1-benzopyran-4-one 2-(3,4 Di methoxy phenyl)-3,5- Di hydroxyl -7-methoxy and, Flavon 4'-OH,5-OH,7-Di-O-Glucoside. The biological effects of these substances are also varied.

CONCLUSION:

Rice bran is an excellent source of nutrients and health benefits. So, it's necessary to carried out one of the stabilizing treatments to inhibit the lipase activity which cause a rancid flavour and it is not appropriate for human use. Also, it could be concluded from this study that the dry heating was the best treatment because of increasing the antioxidant activity, the phenol and flavonoids contents. Besides lowering the free fatty acids in the extracted oil. For that, potentially we can use rice bran extract as a natural preservative safe in the food industry.

Author contribution:

Aliaa M. Abd El-khalek : visualization, software, data curation, and methodology; **Hani M. A. Mohamed**: Supervisor - writing—original draft preparation and investigation; **El- Soukkary, F.A .H.**: methodology, software, writing and validation; **Hanaa M. Hassan**: conceptualization, investigation, methodology, formal analysis, data curation, writing (original draft preparation), writing (review and editing, and visualization).

Table 1. The impact of defatting and stabilisation techniques on the chemical composition of stabilized rice bran

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibers (%)	Carbohydrates (%)
(Un-RB)	9.28±0.07	10.64±0.62	23.10±0.35	8.16±0.05	7.50±0.32	41.31±.63
(DH-RB)	2.53±0.18	12.81±0.48	22.40±0.04	8.82±0.06	12.67±1.54	40.77±.1.9
(A-RB)	9.96±0.17	12.71±0.43	23.61±0.05	8.08±0.005	8.58±0.20	37.05±.57
(De-Un-RB)	9.62±0.18	16.11±.82	0.09±0.05	9.38±0.17	8.67±0.51	56.13±.1.1
(De-DH-RB)	8.55±0.00	17.00±0.08	1.78±0.16	9.94±0.16	9.50±0.18	53.24±.52
(De-A-RB)	11.38±0.02	17.32±0.50	0.29±0.10	9.64±0.04	9.05±0.33	52.33±.86

Each value is expressed as the mean± SD (n= 3)
(Un-RB)Untreated rice bran , (DH-RB)Dry-heating rice bran , (A-RB) Autoclaving rice bran, (De –Un-RB) Defatted Un Treated rice bran, (De- DH-RB) Defatted dry-heating rice bran, (De –A –RB)Defatted autoclaving rice bran.

Table 2. Total Phenolic Compounds and Total Flavonoids of defatted Rice Bran.

Treatment	Phenolics (mg GAE /g extract)	Flavonoids (mg QE/g extract)
(De-Un-RB)	38.83±0.76	8.6±2.42
(De-DH-RB)	40.50±o.50	9.93±0.11
(De-A -RB)	39.75±0.25	4.96±0.05

(De-Un-RB): Defatted Un Treated Rice Bran, (De-DH-RB): Defatted Dry- heating Rice bran and (De –A –RB): Defatted Autoclaving Rice Bran.

Table 3. Antioxidant Activity of Rice Bran Extracts.

Treatments	DPPH		FRAP	ABTS
	% Inhibition IC_{50}(µg/m)		(µM TE/mg sample)	(µM TE/mg sample)
(De-Un-RB)	97.80	37.16	135.07±3.08	189.85 ±10.23
(De-DH-RB)	98.99	36.78	195.07± 8.02	284.46±18.88
(De-A -RB)	98.40	36.79	124.89 ±7.41	210.21 ±9.62

***The IC_{50} values represent the amount of extract needed to remove 50% of radicals from the reaction mixture*.** (De-Un-RB): Defatted Un- Treated rice bran, (De-DH-RB): Defatted dry- heating rice bran and (De-A -RB): Defatted autoclaving rice bran.

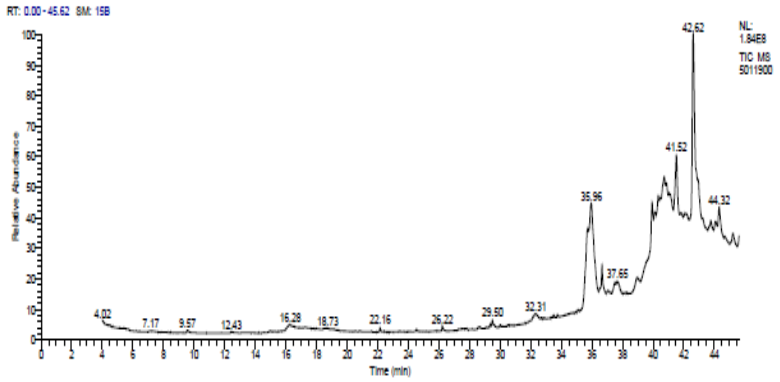
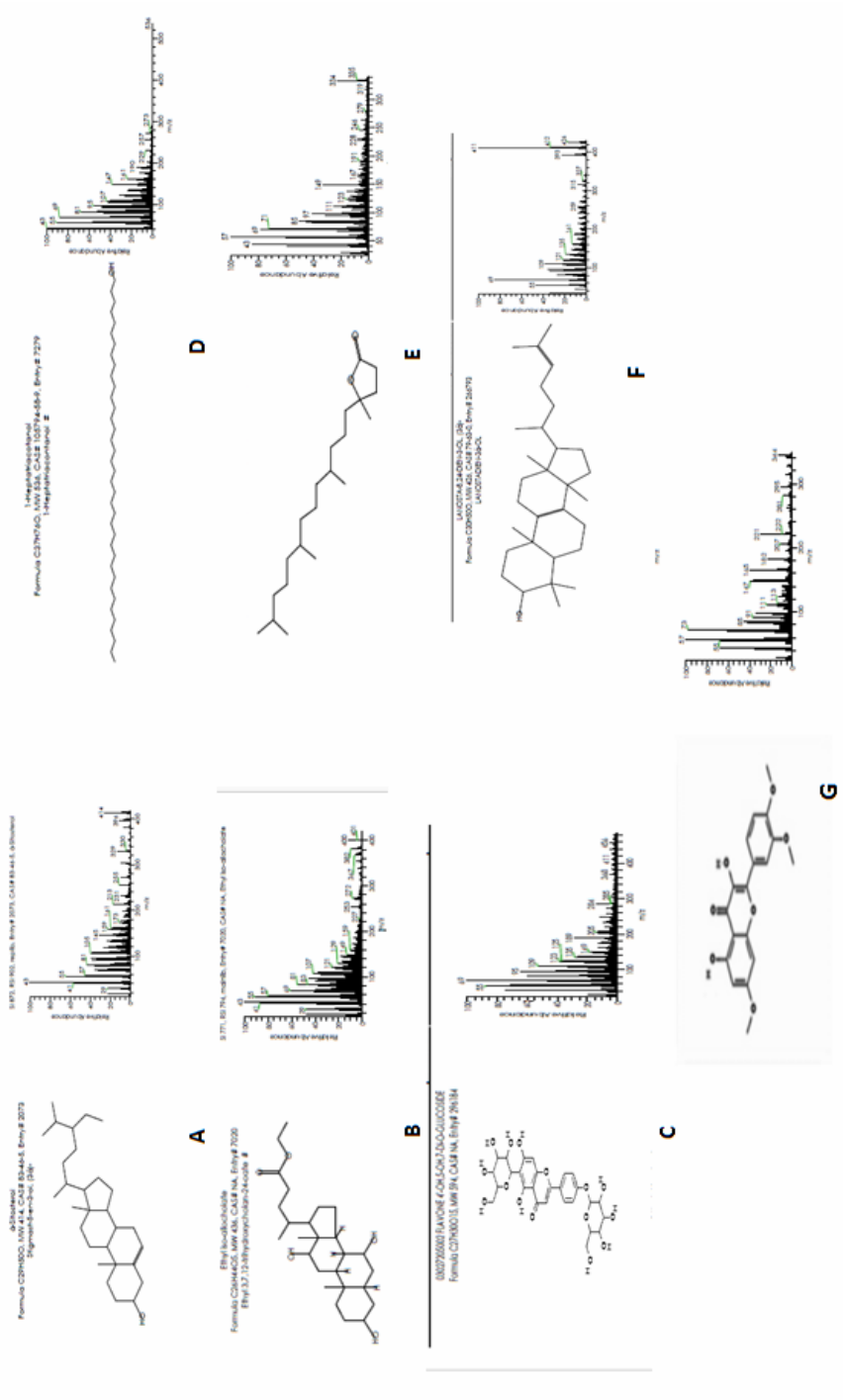


Figure 1. GC-MS Chromatogram for Separation of dry heating Rice bran extract.

Table (4). Show the major phytocomponents of defatted dry heating Rice bran extracts using gas chromatography- mass spectrometry (GC-MS)

NO	RT	Compound Name	Molecular Formula	Area %	MW	Nature	Activity
A-	35.98	ά-Sitosterol	$C_{29}H_{50}O$	10.01	414	natural product	ά-Sitosterol is well known for lowering cholesterol, reducing inflammation, and regulating benign prostrate enlargement. (Muthiah et al., 2017)
B-	38.92	Flavon 4'-OH,5-OH,7-Di-O-Glucoside	$C_{27}H_{30}O_{15}$	1.35	594	Flavonoids	Antioxidant activity (Jitareanu et al., 2013)
C-	39.93	Ethyl iso - allocholate	$C_{26}H_{44}O_5$	5.09	436	steroidal derivative	anti-inflammatory, anticancer, antimicrobial, antiasthma and diuretic properties (Halliwell,1995; Huang et al., 2005)
D-	40.88	1-Heptatriacotcanol	$C_{37}H_{76}O$	2.11	536	natural product (Alcohol)	Lupeol, an antibiotic, has actions that include those against cancer, protozoa, chemoprevention and inflammation, antimalarial, antiviral, antiprotozoal, antitumor and anticancer, enzyme inhibitor, and effects against hypercholesterolemia. (Baskaran and colleagues, 2015).
E-	41.52	Isochiapin B	$C_{19}H_{22}O_6$	9.27	346	sesquiterpene lactone	Anti-insect, antimicrobial, antioxidant, anticancer (Senthilkumar, 2012)
F-	42.61	Lanosta-8,24-dien-3-ol, (3á)-	$C_{30}H_{50}O$	26.54	426	tetracyclic terpenols	Anti-mosquito larvicidal activity, anti-inflammatory, anticancerigenous and analgesic agents (Al-Marzoqi et al., 2016 and PIanowski et al., 2008).
G-	44.32	4H-1-benzopyran-4-one,2-(3,4Di methoxy phenyl)-3,5- Di hydroxyl -7-methoxy	$C_{18}H_{16}O_7$	4.06	344	natural product	Antioxidant, antimicrobial, cancer enzyme inhibitors in pharmaceutical, cosmetics, and food industries (Huang, D. and Irwin, G., 2006)



A. Structure of ά-Sitosterol present in *Rice bran* with RT= 35.70 using GC-MS analysis.
B. Structure of Ethyl iso-allocholate present in *Rice bran* with RT= 39.93 using GC-MS analysis
C. Structure Flavon 4'-OH,5-OH,7-Di-O-Glucoside present in Rice bran with RT= 38.92 using GC-MS analysis.
D. Structure of 1-Heptatriacotanol present in *Rice bran* with RT= 40.88 using GC-MS analysis
E. Structure of Isochiapin B present in *Rice bran* with RT= 41.52 using GC-MS analysis.
F. Structure of Lanosta-8, 24-dien-3-ol, (3á) present in *Rice bran* with RT= 42.61 using GC-MS analysis.
G. Structure of 4H-1-benzopyran-4-one 2-(3,4 Di methoxy phenyl)-3,5- Di hydroxyl -7-methoxy present in *Rice bran* with RT= 44.32 using GC-MS analysis.

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الملخص العربي

تقدير إجمالي محتويات الفينول ، الفلافونويد ، النشاط المضاد للأكسدة و كروماتوجرافيا الغاز- التحليل الطيفي للكتلة لنخالة الأرز الخام والمستقرة منزوعة الدهن

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المنتج الثانوي لعملية طحن الأرز، والذي يستلزم تحويل الأرز البني إلى أرز مصقول هو نخالة الأرز. ويحتوي على عدد كبير من المواد النشطة بيولوجيًا المفيدة. هدفت الدراسة الحالية إلي تقدير النشاط المضاد للأكسدة والتحليل الكروماتوجرافي الغازي لثلاث معاملات للمستخلص الإيثانولي لنخالة الأرز وهم نخالة الأرز منزوعة الدهن الغير معاملة ، نخالة الأرز منزوعة الدهن والمعاملة بالتسخين الجاف ، ونخالة الأرز منزوعة الدهن والمعاملة بالاتوكلاف. تم تقدير ثلاث تقنيات مختلفة مضادة للأكسدة (DPPH، FRAP وABTS)، وكشفت نتائجنا أن مستخلص عينة نخالة الأرز منزوعة الدهن والمعاملة بالتسخين الجاف أظهر أقصى قدر من التثبيط، يليه مستخلص ونخالة الأرز منزوعة الدهن والمعاملة بالاتوكلاف. وأدنى مستوى ظهر في مستخلص نخالة الأرز منزوعة الدهن الغير المعاملة. المعاملة الأكثر فاعلية لمستخلص نخالة الأرز هي نخالة الأرز منزوعة الدهن والمعاملة بالتسخين الجاف في إجمالي المركبات الفينوليه، الفلافونويدات والنشاط المضاد للأكسدة مقارنة بالمعاملات الأخرى للدراسة، وتم اكتشاف وجود سبعة مكونات كيميائية نباتية مختلفة، لكل منها تأثير بيولوجي إضافي تم تقديرها بواسطة التحليل الكروماتوجرافي الغازي ومنهم

Lanosta-8, 24-dien-3-ol, (3á).Ethyl iso-allocholate و ά-Sitosterol, Isochiapin B