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RESEARCH ARTICLE

ASSESSMENT OF PHYTOCHEMICALS, α-TOCOPHEROL, β-CAROTENOID AND ANTIOXIDANT POTENTIAL OF WHEAT BRAN (*TRITICUMAESTIVUM*).

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Abstract

Wheat is one of the most important grain and dietary fiber across the worldand majority of its valuable antioxidant compounds are present in the germ/bran fractions.Wheat bran,by-product of wheat milling industry, holdsmany nutritionally beneficial phytochemicals with antioxidant properties. The current study was to investigate the total phenolic content, tocopherol content, and carotenoid profile for the phytochemical composition of wheat bran. DPPH free radical scavenging test was also performed for the measurement of antioxidant activity of wheat bran extract.For qualitative phytochemical screening, some chemicals standard tests were done which showed the presence of tannin and polyphenols, flavonoid, and saponin in the extract.In quantitative determination, total phenolic content (TPC) in methanolic extract weredetermined as8.97% of gram equivalent of gallic acid.Here we studied a high-performance liquid chromatography (HPLC) technique for the determination of the content of tocopherols and carotenoid in the extract. In this study the concentration of β-carotene in bran sample was 0.11µg/gmand value of tocopherol was 1.77µg/gm. The extract showed the presence of bio-active components which are kinetically active scavenger against DPPH under the testing conditions and accountable for the antioxidant activity.Results indicated that the wheat bran extract have potentials for serving as dietary sources of natural antioxidants and phenolic acids, and may contribute to total dietary tocopherols and carotenoids.

Introduction:-

*Triticumaestivum,*wheat is a major agricultural food crop and dietary constituentacross the world. It has been showed by increasing evidence that wheat and wheat based-products haveexpressive level of phytochemical and natural antioxidants providing health benefits to consumers along with energy and general nutrients.(1,2)

Wheat flour, extracted product of the milling process, contains starchy endosperm which is the main economic and mass component of wheat grain. Bran, germ and pollard are the other portions extracted in the mill stream. (3)The bran layer of the grain is essentially a barrier that protects the germ and endosperm from bacterial, fungal, viral and insect attack.(4)

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Wheat bran, produced by milling, mainly composed of cell wall material rich in many nutritionally valuable constituents, such as dietary fiber, vitamin E, proteins and phenolic compounds with antioxidative properties. (5)However, processing conditions and food matrix affect the bioavailability of nutritional factor of wheat bran. Some of its valuable dietary constituents retained when bran is used in the production of whole meal and brown flours.Sometime these portions are removed in the refinement of white flour resulting in the depletion of these nutrients.Furtherstudies haverevealed that it is the wheat bran fraction (aleurone layer) that contains phenolic acids, carotenoids and tocopherols which consistently retain its radical scavenging and chelating capacities. (6,7,8,9)

It has been proposed that the antioxidant phytochemicals present in wheat bran fractions may control cellular oxidative status of cell and protect biologically important molecules such as DNA, proteins and membrane lipids from oxidative damage, and subsequentlydecrease the risk of several chronic diseases cancer and cardiovascular disease (CVD).(1).Additionally, phenolic compounds found in the wheat bran have been shown a potential role in CVD bypreventlow density lipoprotein (LDL) oxidation, hinder platelet binding to fibrinogen, stimulate thromboxane production and thus prevent triglyceride formation.(10, 11, 12)

Therefore it holds a great potential for food applications. Though, only a little percentage of the produced wheat bran is consumed as a food supplement.(13) Thus, agro and food industry is looking for more valuable end-uses.

With this background, the current study designed to determine the profiles of total phenolic content, tocopherol content, and carotenoid content for the phytochemical composition of wheat bran fraction. The present research also aimed to examine the antioxidant propertyof the bran fraction for the utilization as an antioxidant source.

Materials and Methods:-

Collection of Plant Material:-

Wheat varietywas collected from National Seed Corporation Ltd., Jaipur. Plant documentationcorresponding to this seed was identified similar tovoucher specimen no: RUBL-16133, preserved in Herbarium collection of University of Rajasthan, Jaipur. Production of wheat bran was carried out by milling and separation followed by drying at room temperature in the shade, and then powdered.

Extraction and Testing Sample Preparation:-

100gwheat bran sample was crushed to fine powder (100 mesh micron) using amicro-mill manufactured by Pin Mill Manufacturer (India) and subjected to treatment with 40 mL of 50% methanolfor 12 h at optimumtemperature. The methanolic extract was kept at room temperature until further evaluation of carotenoid, tocopherol, TPCs andantioxidant properties.

Qualitative Phytochemical Screening:-

The dried powdered form of wheat bran were subjected for the preliminary screening and identification of bioactive chemical components like flavonoids, alkaloids, tannins and polyphenols, glycosides, saponins, phenolic compounds, phytosteroids, amino acids and sugar in themedicinal plants according to standard procedures as described by Lespagnol [14]; Harlay et al. 15] and Paris &Moyse [16].

Total phenolic content:-

The total phenolic content of wheat bran extract was determined using Folin-Ciocalteu method. The total phenolic content was expressed as gramsequivalents of gallic acid (GAE) per 100 g extract. Gallic acid was used as standard. In brief, the reaction mixture contained50 µL of wheat bran extracts, 3 mL of pure water, 250 µL of the Folin-Ciocalteu reagent prepared in laboratory, and 0.75 mL of20% sodium carbonate. After 2 hour of reaction at optimum temperature,the absorbance at 765 nm was measured to calculate thephenolic contents in wheat bran using gallic acid as a standard. TheFolin-Ciocalteu reagent was formulated by refluxing a mixture of 85% phosphoric acid, sodiummolybdate, sodium tungstate, and concentratedhydrochloric acid for 10 h and followed by reacting with lithiumsulfateand oxidizing by a few drops of bromine. The resultingsolution was filtered and ready for testing.

Carotenoid Estimation:-

Carotenoids were extracted and analyzedusing HPLC-diode array detection-electrospray ionization (ESI)-tandemmass spectrometry method (14, 15). Briefly, 10g of the groundwheat bran sample was extracted with 30 mL of acetone. 5mL of 0.1% BHT (butylated hydroxyl toluene) in acetone was added in the mixture. The resulting

extraction mixture was subjected to filtration by buchner funnel at room temperature. Aftercentrifugation, the supernatant was filtered and washed 2 times with acetone still residue get colorless. Then filtrate added with anhydrous sodium sulphate. It was filtered again and then reduces the volume in rotatory evaporator. Then dried and weighed. Took all the dry matter in (20:80) DM water and volume was made up to 100mL with acetone.

Instrumentation:-
HPLC analysis wasperformed using a GCmsmadzu TSQ-8030 mass spectrometer (GC 2101 Plus) equipped with an ESI interface and Thermo scientific USA (Ultimate 300 series) HPLC system. HPLCseparation was accomplished according to a previously describedprotocol with modifications (15). The HPLC was performed using aC18 column of Dionex 120A, 250mm x 4.5 mm, 5µ particle size, at room temperature.

Chromatographic Conditions:-
The elution mixture was acetonitrile: dichloromethane: methanol (70:20:10; v/v/v) and the flow rate was 2 mL/min. the run time was 15 min and the wavelength for detection was 452 nm. Identification of carotenoids was accomplished by comparing the HPLCretention time of thesample peaks with that of the authorized pure commercial carotenoidcompounds. Data were acquired with Chromeleon (c) DionexVersion 6.80 SR12 Build 3578 software.The quantification for each carotenoid compound was conducted usingthe total ion counts with an external standard.

TocopherolEstimation:-
Finely milled 5g sample was extracted with 50 mL of 3% ethanolic/ methanolic KOH placed in a brown flask with a screw cap to avoid unnecessary exposure to light and air. The mixture was refluxed on water bath for 30 minutes. Mixture was cooled and separated three times with petroleum ether in separating funnels. Ether layer was washed two times with DM water. DM water layer was discarded and petroleum layer was collected in a beaker. Completely evaporate the ether payer in water bath at 100˚C for 5-10 minutes. Dissolved residue in 5mL of methanol HPLC grade and then sonicated. Filtration of extract was performed before injecting onto the HPLC column.

Instrumentation:-
A Thermo scientific USA, high-performance liquid chromatograph, Ultimate 300 series with variable wavelength detector was used. The detector was set to an excitation wavelength of 290 nm and an emission wavelength of 320 nm.

Chromatographic Conditions:-
HPLC separation was performed using a C18column of Dionex 120A(Agilent Technologies), 250mm x 4.6mm, 5 µm particlesize, at room temperature. The tocopherols were eluted using a mobilephase consisted of methanol and water (97:3) at a flow rate of 1.0 mL/min with run time 10 min. The identification of tocopherols was accompanied by comparative analysis of theHPLC retention time and sample peaks with thatof the certified commercial tocopherol compounds. The quantification for α-tocopherol was conducted using total ion counts with thestandards.

Radical DPPH Scavenging Activity:-
Antioxidant activity of the wheat bran extract and standard was measured on the basis of the radical scavenging effect of the stable DPPH free radical. The working solutions of the wheat bran extractwas prepared using methanol. Gallic acid ranging from 1 to 50µg/ml was used as the standard. 0.135mM DPPH solution in methanol was prepared. Then 2 ml of this solution was mixed with 2 ml of extract solution (ranging from 50µg/ml to250 µg/ml) and the standard solution to be tested separately. The solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using a Thermo Scientific Evolution seriesspectrophotometer against methanol. The control was used is 2 ml of methanol with 2 ml of DPPH solution. The optical density was noted and percentage of inhibition was calculated using the formula given below:

% of Inhibition = (A of control – A of test)/A of control x 100, where A is absorbance

Results and discussion:-

Qualitative phytochemical screening:-
Phytochemical components in the plants are identified to be biologically active complexes and accountable for various activities such as antimicrobial, antioxidant, and anticancer [17]. In the present study, the phytochemical screening of wheat bran extract had been performed with five solvents: hexane, chloroform, ethyl acetate, methanol

and water. The extractive values in each solvent were as follows: hexane-1.54%, chloroform-0.15%, ethyl acetate-0.61%, methanol-5.5% and water-6.9%. Results had shown the presence of few phytoconstituents in particular solvent; the summary of the results are presented in table 1.

Table 1. Phytochemicals detected in Wheat Bran Extract

S.No.	Phytoconstituents	Results	Test Method
1.	Alkaloids	Absent in All extracts	API 2008
2.	Glycosides	Absent in All extracts	API 2008
3.	Saponins	Present in Water extract	API 2008
4.	Tannins & Polyphenols	Present in Methanolic Extract	API 2008
5.	Steroid	Absent in All extracts	API 2008
6.	Flavonoids	Present in Methanolic Extract	API 2008
7.	Amino Acids	Absent in All extracts	API 2008

API: Ayurvedic Pharmacopeia of India

Amount of Total Phenolic contents:-

The organizedresearch pertaining to this investigation shows that the plant phenolics constitute majority of compounds acting as free radical scavengers or primary antioxidants. [18] Therefore, it is worthwhile to determine their total amount in tested extracts. Based on the absorbance value of the extract solution reacted with Folin-Ciocalteu's reagent, the total phenolic content in the extract of wheat bran were determined as 8.97% of gram equivalent of gallic acid.

Estimation of β-carotene:-

β - Carotene is generally known as a biological radical scavenger and play asignificant role in initial stages of lipid peroxidation inhibition. The β - carotene content of wheat bran extract is nutritionally important as a daily dietary component. [18]The chromatogram and the content of β-carotene in wheat bran extractare shown in Fig. 1. Carotenoids identification was done by comparison with a pure standard (β-carotene) and based on retention times. The amount of β-carotene was determined as 0.11µg/gm under the experimental conditions.

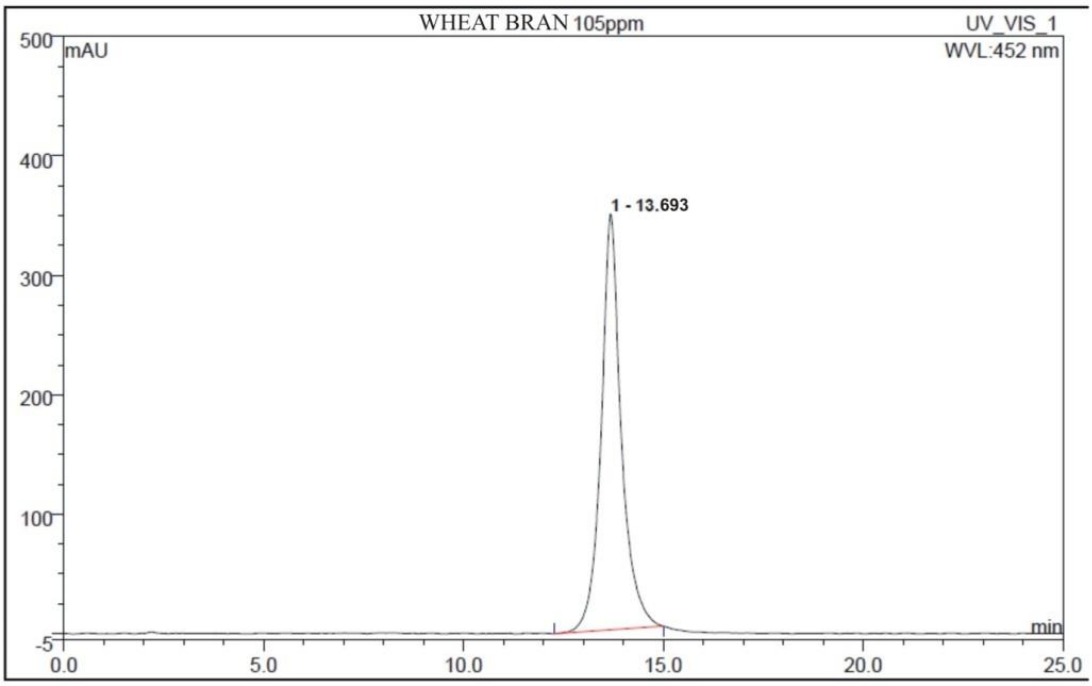

Fig1:- Chromatogram for β-carotene in wheat bran extract.

Estimation of α-tocopherol:-

The tocopherolcontent in wheat bran extract wasdetermined by retention time and calculated by using linear regression from the calibration curve of the standard solution. In this study, HPLC method was used for the quantitative determination of α-tocopherol in extract, accomplished by a comparison of retention times and areas with that of standard α-tocopherol. The amount of α-tocopherol extracted for wheat bran was determined as1.77µg/gm under experimental conditions.

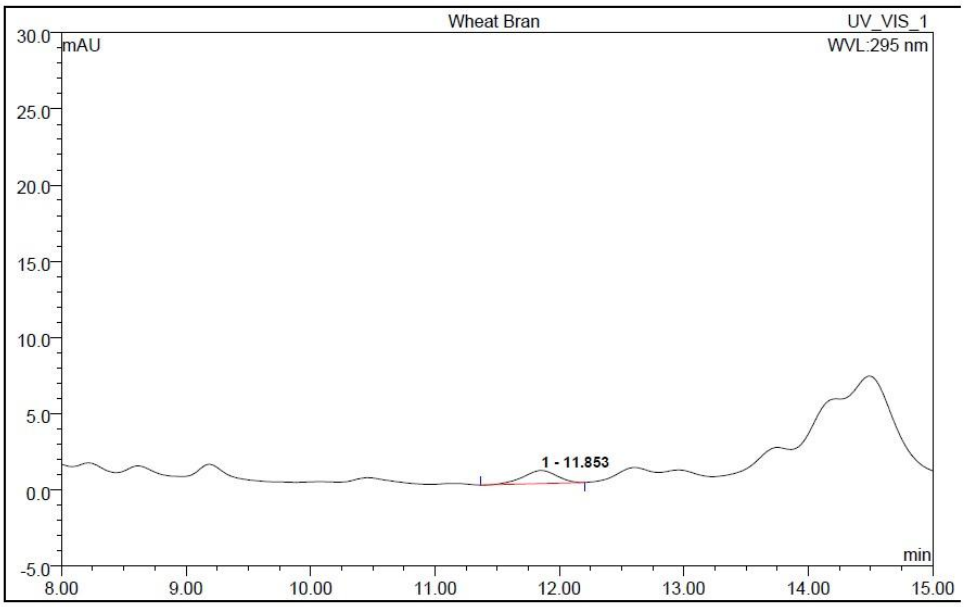

Fig 2:-Chromatogram for the α-tocopherol in wheat bran extract

DPPH Assay (Radical Scavenging Activity):-

DPPH radical scavenging activity assay is performed to examine the ability of compounds to act as hydrogen donors or free radical scavengers and to calculatethe antioxidant activity of plant extract [19]. The ethanol extracts of wheat bran sample was analyzed for their IC50 values against DPPH (**Figure 1**). IC50 is the required concentration of wheat bran antioxidants to scavenge 50% DPPH radicals in the reaction mixtures. The free radical scavenging property was calculated and expressed as mg/mL of TPC. As shown in Table 2 and Fig.3, the percentage of inhibition increases with increase in concentration of wheat bran extract proved that extract possessed high antioxidant activity which augmented in dose dependent manner. These data indicated that the wheat bran extract is kinetically active scavenger against DPPH under the testing conditions.

Table 2:- DPPH Free radical scavenging activity of Wheat Bran.

Sample	Concentration (ml)	% Inhibition	IC50 (mg/mL)
Wheat Bran	0.5	18.6	0.6678
	1.0	47.24	
	1.5	64.07	
Control Absorbance = 0.618			

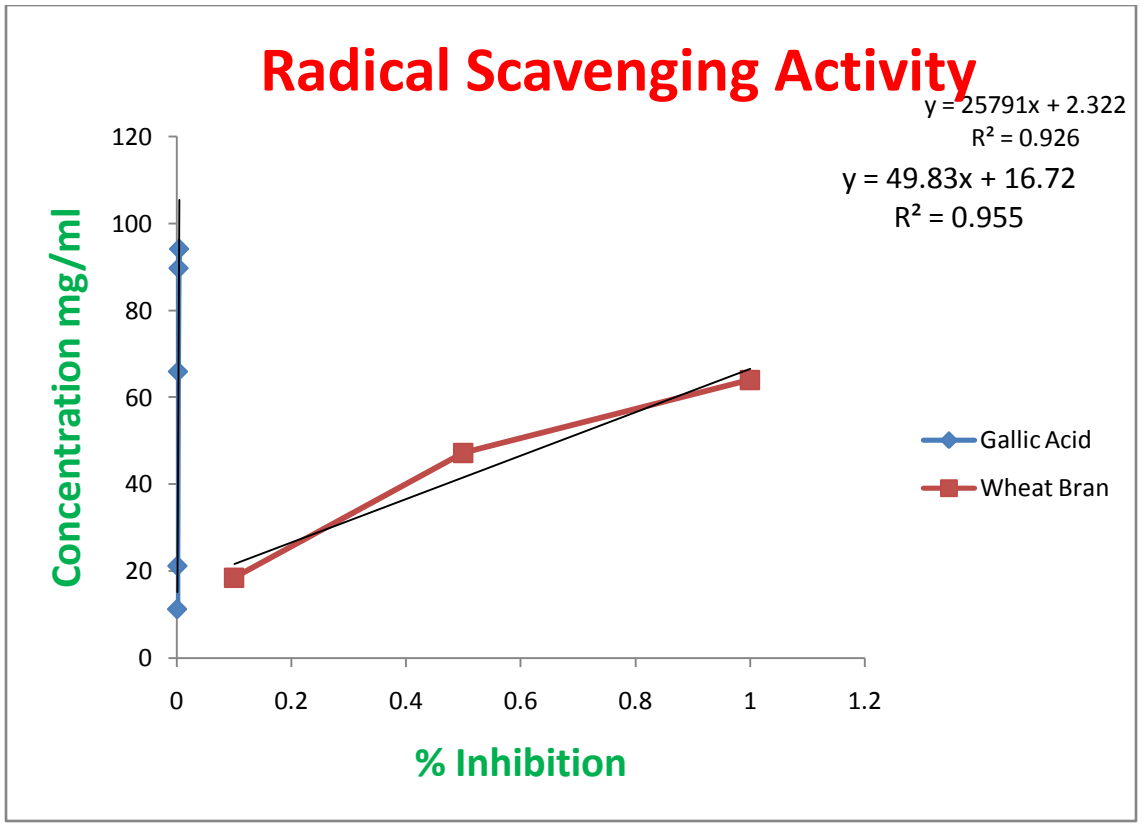

Fig 3:-Graphical representation of DPPH Scavenging activity of Wheat Bran Extract.

Conclusion:-

From the above-mentioned, it may be concluded that the extract of wheat brandemonstrated potent antioxidantand an exceptional source of phytochemicals. Preliminary phytochemical analysis revealed thepresence of tannin, polyphenols, flavonoid, and saponin in wheat bran extracts suggested that this extract could be a potential source of natural antioxidant having great importance in improving the wellbeing of the consumers as a result of the presence of severalconstituents that are vital for good strength. The results obtained from HPLC analysis confirmed the presence of adequate amount of β-carotenoid and α-tocopherol in theextract contributes to total dietary carotenoids and tocopherols. These findings candidate the plant as a noble objective for more in-depth studies which may be of great significance when discussing health concerns of grain products.

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