INVESTIGATION OF IN VITRO ANTIOXIDANT PROPERTIES OF NANOPARTICULATED ANTHOCYANINS ISOLATED FROM SORGHUM BRAN

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Abstract

Over the past decade, there has been an increasing interest in using nanotechnology for cancer therapy. The development of smart targeted nanoparticles (NPs) that can deliver drugs at a sustained rate directly to cancer cells may provide better efficacy and lower toxicity for treating primary and advanced metastatic tumors.There is an increasingly aware of diet related health problems and therefore demanding natural ingredients which are expected to be safe and health-promoting. Recently, number of studies on health benefits associated with natural phytochemicals have been demonstrated. The present study identifies the antioxidant potency of nanoparticulated anthocyanins from Sorghum bran were investigated by employing various established *in vitro* systems, such as reducing power, DPPH/superoxide/hydroxyl radical scavenging, ferrous ion chelation and lipid peroxidation. The results proved that the anthocynanins can act as lead molecule towards mitigation of various human aliments by its antioxidant property.

Key words: Sorghum bran, Anthocyanins, Nanoparticles, Antioxidants.

Introduction

Medicinal plants have been used since from the beginning of human life and these form the basis for the development of modern medicine .Medicinal plants usage for human is a result of many struggles against illness from which the mankind learned to pursue drugs in bark, seeds, fruit bodies and other parts of plants (Bilijana Bauer *et al.*, 2012).Medicinal plants are the main resources in almost all the cultures by ensuring its safety, quality and effectiveness. Development of novel drugs from herbal drugs can help in the healthcare system to treat various human diseases in future (Fatemeh Jamshidi Kia *et al.*, 2018). The plants naturally produce secondary metabolites from which the anti-cancer activities can be investigated and this leads to the development of new clinical drugs. Various technologies have been developed particularly nanoparticles for nanomedicines which enhance anticancer activities of derived drugs by controlling the release of compound and investigating a new methods for administration. Many natural products and their analogues have an potent anticancer agents and anticancer properties of various plants or phytochemicals are being identified. The main living traditions that still exists even today is

traditional Indian medicine. Ayurveda and the traditional Chinese medicine which knit together the diverse knowledge of therapeutic plants (Uttpla anand *et al.*,2019).

Sorghum is a the world's fifth largest produced cereal after maize, rice, wheat and barley. In Africa it is the second produced cereal after maize and the production of the sorghum is almost one half of the world crop production. Sorghum contains a phenolic compounds in the form of phenolic acids, flavonoids and condensed tannins which have antioxidant activity. It is genetically diverse compound and some species have high amount of polyphenols (Haroon Khan, 2014).It is also reported that high amount of polyphenols components are with strong anticancer activity Many of the grains are not evaluated in the health promotion potential even though there are approximately 40,000 of sorghum accessions. In the traditional medicines sorghum based foods such as teas, beers and extracts are finding an importance on understanding the increased biological effects of sorghum (Young Min Ham *et al*., 2019).

Almost all kinds of phenolics are found in sorghum and much more are present in their outer layer (bran. A high concentration of proanthocyanidins s present in Sorghum bran, which reduces the risk of certain types of cancer. Anthocyanin is only known for the coloring properties and now anthocyanin plays an important role in the health benefits as dietary antioxidants by preventing neuronal diseases, cancer, diabetes, cardiovascular illness, inflammation and many other diseases. Anthocyanins are the poly phenolic pigments that belongs to a flavonoid group and it is responsible for the red-orange to blue-violet colours present in plant organs. Epidemiological studies suggested that the high consumption of anthocyanin may lower the risk of various diseases. The pigments in the anthocyanins are in glycosylated form. Among these cyaniding-3-glucoside is the major anthocyanin found in most of the plants (Xiaoping luo *et al.*,2018).

Nanotechnology in drugs provide a new option for the drug delivery and drug therapies. It has greater potential in increasing the selectivity of the biological compounds approaches for eliciting cancer cell death. Nanomaterials selectively targets the cancer biomarkers and cancer cells allowing early detection ,more sensitive diagnosis and also monitoring of the progress of therapy and tumor burden over time . Cancer nano therapeutics has overcome several limitations of conventional therapies , such as non-specific bio distribution.

The present aims to evaluate the antioxidant efficacy of nanoparticulated anthocyanins extracted from sorghum bran in ivtro .

Methodology

Preparation Of Extract Of The Sample

The Sorghum plant was collected from farming land in Coimbatore. The grains in the plants are removed and the bran is separated from the plant. The obtained bran was powdered. 10g of bran powder were taken in a

conical flask for sample extraction by using 100ml of chloroform solvent for 72hrs.After the incubation, the extract was air dried. The crude that was obtained was dissolved in water and used for studies..

Synthesis Of Zinc Oxide Nanoparticles

In a typical reaction mixture ,5-10 ml of the aqueous extract of red sorghum bran was added to 300 ml of 4Mm of aqueous zinc sulphate heptahydrate solution and stirred at room temperature for 5 min s to achieve the pale yellow solution. After that, 1M sodium hydroxide solution is added to the mixture drop by drop with continuous stirring at room temperature. The yellow color of the above mixture starts change to yellowish suspension at pH 12. The suspended particles were purified by dispensing in sterile distilled water and centrifuged thrice. Further, the white precipitate particles were washed with ethanol to remove the impurities for the final product. The white powder was obtained after drying at 60ºC in vaccum oven for 6 hrs.The formed nanocrytals were confirmed by its UV Vis absorption at 340nms.

***In Vitro* Antioxidant Activity assay of wsorghum Bran Nanoparticles**

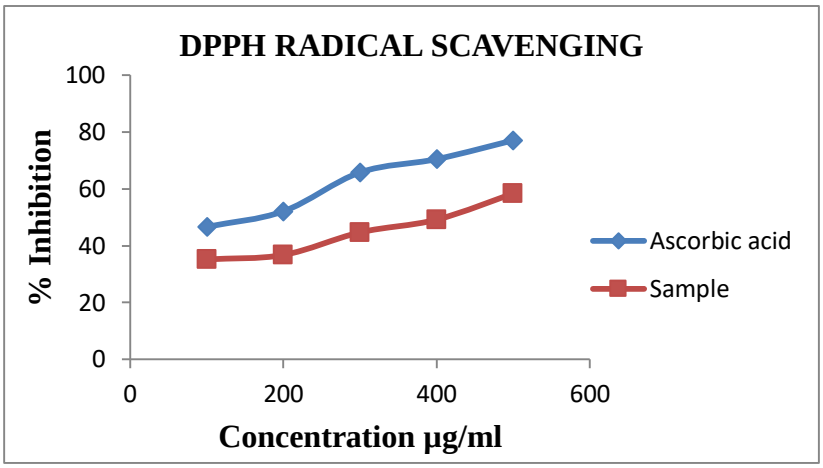
DPPH Scavenging Activity, Hydroxyl Radical Scavenging Assay, Nitric Oxide Scavenging Assay, Reducing Power Activity, Ferric Reducing Antioxidant Power (FRAP) Assay & Total Antioxidant Capacity were assayed by standard methods.

Results and Discussion

DPPH Scavenging Activity

DPPH is relatively stable radical. The assay is based on the measurement of the scavenging ability of the antioxidats towards the stable radical which reacts with suitable reducing agent (Blios *et al.*,2009)

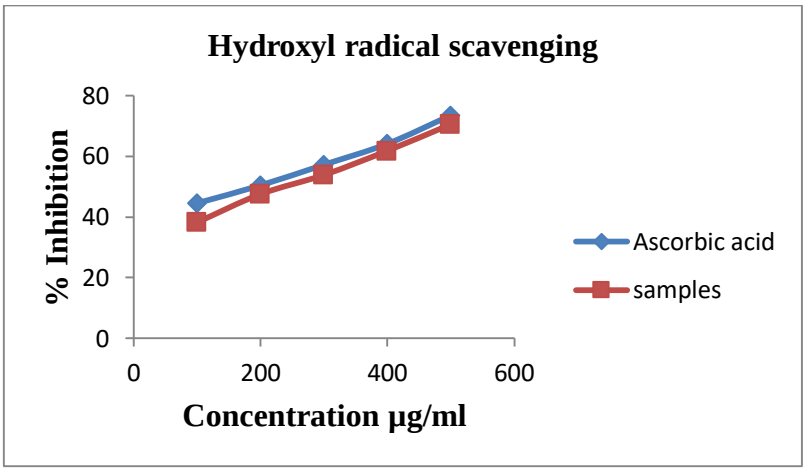
The percntage radical scavenging activity of ZnONP's of red sorghum bran wre determined against DPPH.IC_{50} values are calculated with the regression equation that shows inverse relationship between the IC_{50} value and percentage of scavenging of a sample. The radical scavenging activity for the ZnONP's of red sorghum bran IC_{50}=143.20 µg/ml,IC_{50} for sample is found to be 387.30 µg/ml.The highest scavenging activity of the ZnONP's of red sorghum bran was found to be 77.18±0.96µg/ml for standard and for sample the highest scavenging activity is 58.48±1.71µg/ml at a concentration of 500µg /ml.



Hydroxyl Radical Scavenging Activity

Hydroxyl radical is the most reative oxygen centered species and causes severe damage to adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by generating the hydroxy radical experimentally (Sasikumar *et al* ., 2014).

In this study, the ZnONP's of red sorghum bran showed the concentration dependent scavenging activity of the ZnONP's was found to be73.45±0.92µg/ml for standard ascorbic acid and that of samplewas found to be 70.42±0.89µg/ml at the concentration of 500 µg/ml. The IC_{50} value for the ZnONP's of red sorghum bran and ascorbic acid were found to be 267.89 and 188.07 respectively.

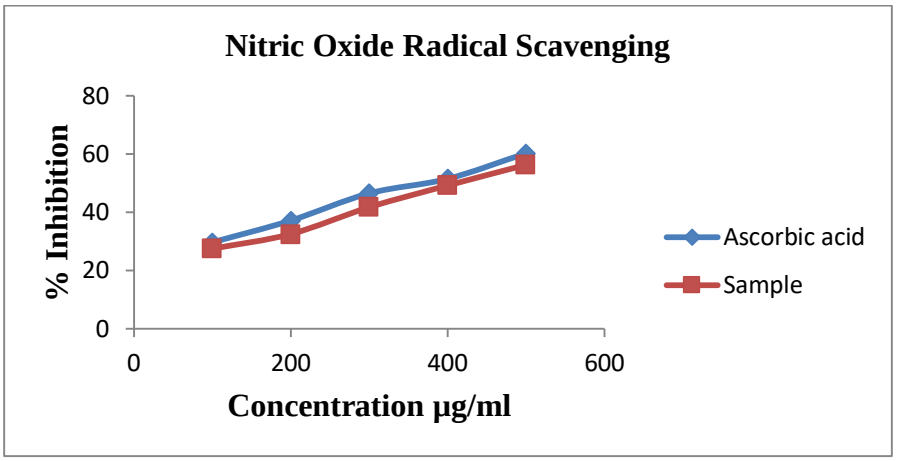


The previous study also revealed that ZnONP's of aqeous and ZnONP's of polypols showed the highest scavenging activity with hydroxyl radical scavenging (50.92µg/ml, 48.73µg/ml) (Siba soren *et al.*,2018).

Nitric Oxide Scavenging Activity

Nitric oxide is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation, anti-microbial and anti-tumor activities. Nitric oxide or reactive nitrogen species formed during their reaction with oxygen or with super oxides, such as NO_2,N_2O_4,N_3O_4 and NO_2 are very reactive (Shreedhara *et al.*,2009).

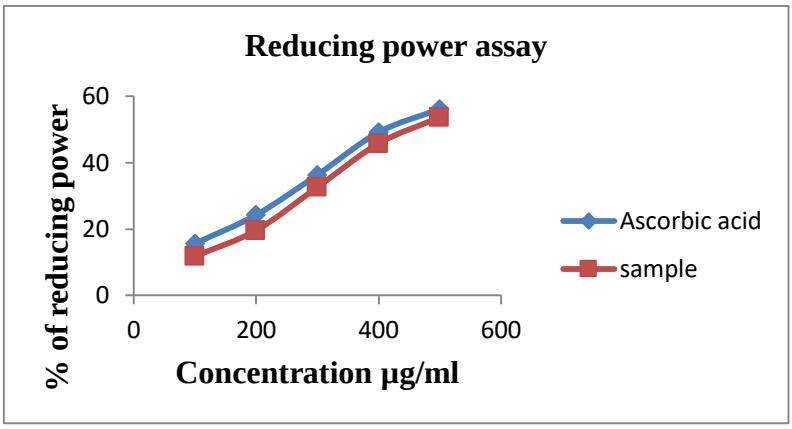
In our present study, the ZnONP's of red sorghum bran showed concentration dependent scavenging activity. The highest scavenging of the ZnONP's of Red sorghum bran was found to be 56.25±0.94µg/ml and that of the standard ascorbic acid found to be 60.31±1.79µg/ml at 500 µg/ml.IC_{50} values of the ZnONP's of red sorghum bran and ascorbic acid were found to be 415.00 µg/ml and 365.63 µg/ml respectively.



Reducing Power Activity

Reducing power is associated with anti-oxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process. The yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each compounds.

The reducing power assay of standard ascorbic acid of ZnONP's of Red sorghum bran depicted the following figure. The maximum inhibition was observed with increasing concentration of ZnONP's of sample to 500µg of standard. Absorbance values maximum value at 40.51±1.28µg/ml and for the standard the maximum concentration is 49.11±0.89µg/ml.

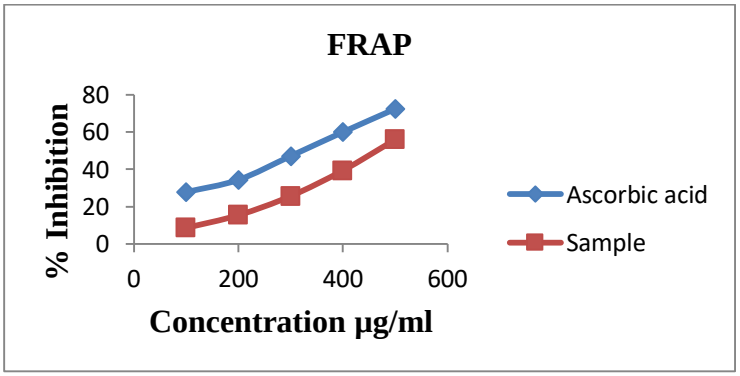


Ferric Reducing Assay Power (FRAP)

FRAP assay has been used as a novel method to determine antioxidant activity and is considered as a useful indicator of the bodys antioxidant status to counteract the oxidative damage due to ROS. Red sorghum bran increases the free radical scavenging activity.

FRAP activity is mainly due to their redox potential which plays an important role in adsorbing and neutralizing free radicals quenching or decomposing peroxides. The results from this study confirmed that the constituents having antioxidant and pharmacological effect of ZnONP's of red sorghum bran. Higher FRAP values denotes higher antioxidant capacity because FRAP value is based on reducing ferric ion, whereas the antioxidants are the reducing agents (Louise Svensson, 2010).

Ferric reducing power of standard ascorbic acid and ZnONP's of Red sorghum bran depicted in the figure. The maximum inhibition was observed with increasing concentration of ZnONP's 500µg . Absorbance values had a maximum value at 55.75±0.93µg/ml and for the standard the maximum concentration is 72.35±0.57 µg/ml.



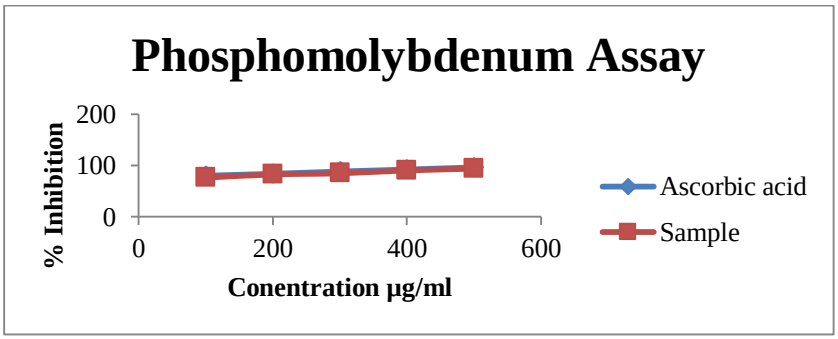
DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY (TAC)

Phosphomolybdenum Assay

Phosphomolybdenum assay which is also known as total antioxidant capacity. It is an analyte frequently used to detect the antioxidant status of bilogical samples and can elevate the antioxidant response against the free radicals in certain diseases (Camila Peres Rubio *et al.*,2016). It is the measure of the amount of free radical scavenged by the test solution.

In general, they measure the low molecular weight chain breaking antoxidants. The assessment of oxidative stress include all these measurements. Low total antioxidant capacity could be increased susceptibility to oxidative damage.

The phosphomolybdenum assay of standard ascorbic acid of ZnONP's of Red sorghum bran represented the following activity. The maximum inhibition was observed with increasing concentration of ZnONP's of sample to 500µg of standard. Absorbance values maximum value at 94.28±1.42µg/ml and for the standard the maximum concentration is 96.44±1.39µg/ml.



There was direct relationship between concentration of all samples. In highest concentration (500µg/ml) amounts of TAC were 0.79±0.06µg/ml,0.68µg/ml and 0.56µg/ml for ascorbic acid ZnONPs respectively.

From our present study it can be concluded, that the nanoparticles of sorghum bram anthocyanins exhibited antioxidant properties, which can be further exploited as potent anticancer drug. This tudy will form a basis for the identification of lead compounds targeted towards cancer with high efficacy and low adverse effects.

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