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Health Preserving Potential of Shea Germinated Nut, a Process Reject: Antioxidant Activity of Protein and Hydroalcoholic Extracts

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Abstract
Shea (*Vitellaria paradoxa*) is a Sapotaceae which leaves, roots and nuts butter are widely used in traditional medicine for the treatment of several health disturbances. Here the healing potential of its germinated nuts was demonstrated *in vitro*, through the antioxidant power of their crude/dialysed proteins and hydroalcoholic extracts; DPPH test was carried out therefore. Both proteins and hydroalcoholic extracts of germinated seeds reduced significantly DPPH, at various rates; hydroalcoholic extract recorded the best rate (86.30%). Crude (31.62%) and fractioned proteins (34%) registered less than the half of hydroalcoholic extracts inhibition rate. Ungerminated seeds crude and hydroalcoholic extract have also reduced the radical DPPH with values of 38% and 86.13% respectively. These results show that the protein and hydroalcoholic extracts of shea seed cakes could be validly exploited in the management or treatment of diseases due oxidative stress.

Keywords: Shea germinated nuts; press cakes; protein and hydroalcoholic extracts; antioxidant power; DPPH

Introduction

The antioxidant power of several plants has helped in explaining their therapeutic potential (Favier, 2003) [5]. Antioxidant substances are hydrogen atom donor compound leading to a stable free radical. They are most often hindered phenols or secondary aromatic amines (Poaty-Poaty, 2004) [12]. They also contribute to stop the generation of free radicals by implementing several mechanisms (Rolland, 2004) [14]. The presence in excess of free radicals causes the appearance of abnormal biological molecules at the basis of oxidative stress. Oxidative stress causes significant damage that accelerates cellular aging. This aging leads to serious health disturbances such as cardiovascular and neurodegenerative diseases, cancer, diabetes, metabolic syndrome and digestive diseases (Aseervatham *et al*., 2013) [2]. Several situations in daily life lead to the production of free radicals responsible for oxidative stress. These include, for example, deficiency of one or more antioxidants provided by nutrition such as vitamins or trace elements (Sohal *et al*., 2002; Favier, 2003) [5] or even environmental exposure to prooxidant factors such as tobacco, alcohol, drugs and pesticides (Sohal *et al*., 2002; Magder, 2006) [17, 9]. To address this problem, antioxidants are advocated, and the most commonly used are synthetic ones. However, the use of available synthetic antioxidant molecules is, currently, questioned due to the potential health risks and toxicity they are capable of causing (Kicel *et al*., 2016; Liu and Yang, 2018) [6, 8]. Therefore, the focus is, increasingly, on finding new sources of antioxidants including medicinal plants (Liu *et al*., 2017; Wang *et al*., 2018) [7, 20]. Thus, shea (*Vitellaria paradoxa*, Gaertn C.F), known for its many therapeutic virtues was the subject of this study. In fact, during the technology of transformation of shea nuts into butter, the germinated seeds are rejected, because they would produce butter of poor commercial quality. However if several studies linked the antioxidant power of plants to phenolic comppounds, in shea protein (sometime), other studies reported the presence of protein and phenolics coumpouds, in shea kernels (Djoman *et al*., 2021). Thus, cakes could the press cakes of germinated shea kernels be a major asset in the reduction of oxidative stress. The objective of this study is to enhance the value of germinated shea seed press cakes by highlighting their antioxidant potential. This was achieved by evaluating the antioxidant activity of their crude and dialysed proteins; so were their hydroalcoholic extractcs. The DPPH free radical scavenging method was used for that purpose.

Material and Methods

Material

Germinated and ungerminated shea nuts constituted the biological material of the present study. Shea nuts were collected under shea trees, in july 2020 during harvrest period. Ungerminated nuts served as control. Pure distilled water both were puchased in a pharmacy officine. Pure distilled water and a semi-permeable hydrophilic membrane with a cut-off diameter of 10-12 KDa (SIGMAALDRICH, USA andCanada), as for them, served for dialysis process.

Methods

Shea Seeds Proteins Extraction

Shea seeds were first, crushed and delipidated with aceton solution; a fatless powder was obtained after rotavaporation. Protein extraction from the previous power, consisted in a solubilization at pH 8 and isoelectric precipitation at pH 4.3, successively (Acosta *et al*., 2016) [1]. Hence, 2.5 g of the delipidated shea seeds powder were suspended in distilled

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water at a ratio of 1:10 (W/V, flour/water). The pH of the suspensions was adjusted to 8 with a 0.1 M NaOH solution. The resulting solution was centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was recovered and its pH was adjusted to 4.3 with a 2 M HCl solution. The final mixture was centrifuged at 10000 rpm for 20 min at 4 °C and the pellet was recovered and resuspended in distilled water (5%).

Preparation of Shea Seeds Hydroalcoholic Extract

1 g of shea seed fatless powder was dissolved in 10 mL of 70% (v/v) methanol. The mixture obtained after homogenization was centrifuged at 1000 rpm for 10 min. The pellet was recovered in 10 mL of 70% (v/v) methanol and centrifuged again. This operation will be repeated a third time. All three supernatants were recovered and stored for analysis (Singleton *et al*., 1999) [16].

Fractionnement of Shea Seeds Protein Extracts

Shea seeds proteins were fractioned by dialysis through a cellophane membrane (10 KD) Hence, 3 mL of protein extract introduced into a dialysis bag. This latest was firmly tied and plunged into a glass bottle containing pure distiller water, for hours. Both inside and outside contents of dialyse bag were concentrated and stored for future assay

Determination of Antioxidant Activity by DPPH

The DPPH test is a colorimetric method based on the reduction of the DPPH free radical. Indeed the reduction of this molecule is characterized by a field of coloration which passes from purple to yellow. The 1,1-diphenyl-2-picrylhydrazyl (DPPH˙) molecule is a stable free radical, whose solution has a violet coloration and a characteristic absorption at 517 nm (Brand-Williams, 1995) [3]. The reaction mixture consisted of 1950 µl of DPPH solution in a tube containing 50 µl of aqueous protein or hydroalcoholic extract. The control sample was the mixture of 50 µl of methanol and 1950 µl of DPPH solution. After incubation for 30 min at 30 °C in the dark, the absorbance was measured at 517 nm against a blank.

Results and Discussion

Results

Antioxidant Activity of Undialyzed Protein Extracts

Results of DPPH free radical inhibition by crude proteins showed that shea germinated seeds reduced significantly the DPPH as ungerminated seeds did. Nevertheless, the latest seeds (38%) a relatively higher inhibition rate than the first ones (31.62%). However, both values were lower than vitamin C one (92.61%).

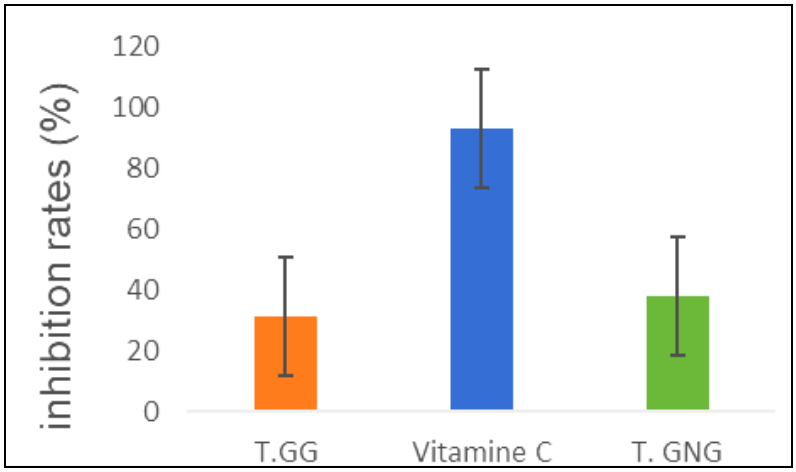

Fig 1: Antioxidant activity of crude proteins

Antioxidant Activity of Dialysed Proteins

About dialyzed proteins, the whole fractions (in the bag internal and out of the bag external), also showed DPPH inhibition, either for germinated or for ungerminated seeds. Whatever, both fractions of germinated seeds registered best inhibition percentages (34% and 20.54%), copared to ungerminated ones (22.56% and 12.56%).

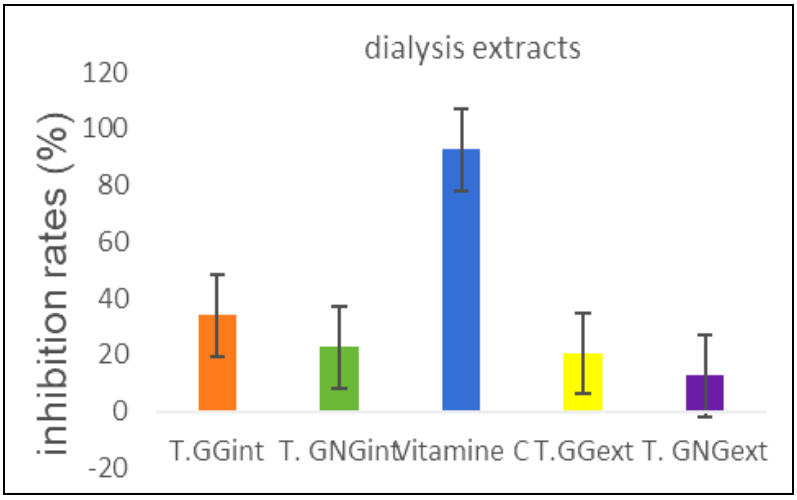

Fig 2: Antioxidant power of dialyzed proteins of germinated and ungerminated shea seed

Antioxidant Power of Hydroalcoholic Extracts from Shea Germminated Seeds Press Cakes

Hydroalcoholic extracts of both germinated and ungerminated shea seed press cakes also exibited ability to scavenge free radicals. Their inhibition rate were significantly similar with 86.30% for germinated and 86.13% for ungerminated sheaa seeds. Vitamin C inhibition rate, as for it remained the highest (92.61%).

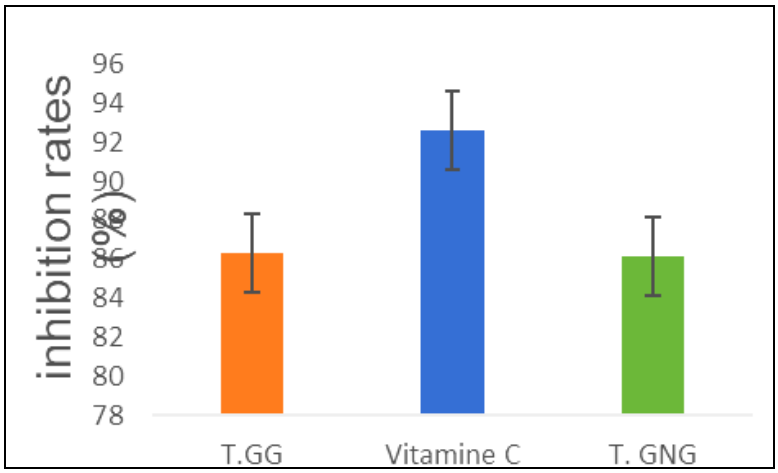

Fig 3: Antioxidant power of germinated and ungerminated shhea seeds hydroalcoholic extracts

Discussion

In recent years, the world of biological and medical sciences has been invaded by a new concept, that of "oxidative stress", that is to say situation where the cell no longer controls the excessive presence of toxic oxygenated radicals, a situation that researchers implicate in most human diseases (Favier, 2003) [5]. In this context, medicinal plants remain the reliable source of active principles known for their therapeutic properties. These plants abound in active molecules such as phenolic compounds and certain proteins or peptides that are powerful antioxidants or excellent free radical scavengers. Here is the case of the antioxidant ability of shea germinated seeds. These latest are generally rejected during shea butter processing because of the bad butter they would generate. Proteins and hydroalcoholic extracts of the cakes resulting from these germinated seeds have demonstrated *in vitro* antioxidant activity. The percentages of inhibition of DPPH by the whole extracts (12.56 to 86.30%) were whatever lower thanthat of vitamin C is 92%. According to Sahgal *et al.* (2009) [15], even a low percentage of inhibition, indicates some proton donation capacity. The DPPH radical scavenging activity of shea protein extracts ranged from 12.56 to 38%. The anti-radical potential of these extracts show that extracts from shea germinated seeds have good activity for both dialysis and undialysis portions than ungerminated ones. This is in conformity with the results of Soro *et al.*, 2014 [18] who stated that the levels of bioavailable lysine, are higher in flours containing germinated soybeans than those containing ungerminated ones. This could be explained by the partial hydrolysis of seeds proteins (storage proteins) during germination (Purchas *et al.*, 2003) [13]. The better inhibition rate of DPPH by the internal dialysate (34%) from germinated seeds, in contrast to the other dialysed extracts, could be explained by the fact that the separation generated two fractions of peptides sizing lower than 10 KDa (external fraction) and more than 10 KDa (internal fraction), and which are both, undowed with bioactive function. Nevertheless, the inhibition rate of internal dialysate (fraction) would suggest a best inhibition potential of peptids sizing more than 10 KDa. Whatever, a best separation would be necessary. Above all, DPPH inhibition by crude proteins of ungerminated and germinated seeds (38% and 31.62%) could suggest, to simply exploit the whole crude protein (without separation) of shea seeds, but fractionment would be the best approach for knowing exactly the sizes of peptids which induce antioxidant ability to shea seeds proteins. About hydroalcoholic extracts of shea seeds, DPPH radical were at far more deeply reduced than proteins; inhibition rates (86.30%) ungerminated (86.13%) values were relatively closed to that of vitamin C (92.61%). Such behaviour would signify that shea seeds hydroalcoholic extracts could be considered as very good antioxidants. These different results could be explained by the presence of phenolic compounds contained in shea seeds hydroalcoholic extract. Indeed, it is admitted that these chemical groups are generally involved in the biological activity of medicinal plants and are recognized as powerful anioxidants (Olasunkanmi *et al.*, 2017) [11]. Therefore, molecules contained in the protein and hydroalcoholic extracts of shea seeds could be considered as valuable antioxidant agents. These extracts could then, be used for therapeutic applications. It is worth underlining here, that antioxidants contribute very effectively to the prevention of diseases such as cancer, cardiovascular diseases and metabolic diseases including diabetes. Hence, either the entire germinated seeds or their protein/hydroalcoholic extracts could be directly consumed as food complement or be involved in pharmaceutical formulations (feeding or cosmetical) for the prevention/treatment of disturbances linked to oxidative stress. It is worth to reassured (readers) by noting that, in shea producing areas, shea seed is sometime, consumed; it should be considered as a tonifiant seed which is frequently eaten during field work.

Conclusion

The objective of this work was to enhance the value of germinated shea seeds which are considered as a reject, by highlighting their antioxidant potential. At the whole, both germinated and ungerminated seeds extracts (protein and hydroalcoholic) presented ability to reduce significantly DPPH. Thus, it was well demonstrated here, that shea germinated seeds which are generally rejected because of the low quality of their butter, have better or at least the same ability to be exploited as bioressources in the prevention/fight against heath disturbances due to oxidative stress. Hence, shea germinated seeds press cakes (powder) could be consumed directly or their protein hydroalcoholic extracts could be

exploited as food additives or pharmaceutical/cosmetical ingredient. For this latest purpose, shea seeds present extracts could be improved with the purification of the active molecules. Hense, research is on to isolate and purify the active constituents of each extracts in order to identify antioxidant molecules and other health benefic molecules.

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