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Mini review

Concentration and Differential fractions of Saponin Rich *Aframomum melegueta* Extract Influences In-Vitro Antioxidant Activities, and Silver Nitrate can Stimulate such Activities in the Plant

John Ajayi*, Ibraheem Omodele

Department of Biochemistry, Federal University of Technology, Akure, Nigeria

ABSTRACT

Free radicals are generated during normal metabolic activities of the body, and are capable of causing damage to cells and tissues when their rate of production exceeds their removal from the body, a pathologic condition commonly referred to as oxidative damage. Plant secondary metabolites, are proved to be effective in scavenging such free radicals. The plant saponin extracts were prepared using different solvent extraction fractions. It was intended at determining what fractions of the solvents would be best suitable for the extraction of the plant's saponin rich fraction. Different concentrations were then prepared from the stock saponin solution, to evaluate the most potent concentration. Total phenolics, antioxidant activity using DPPH, chelating activity, hydrogen peroxide scavenging activity and the effect of silver nitrate on the plant's activity were then evaluated.

The 80% saponin fraction at the 150µg/ml concentration demonstrated the highest antioxidant activities using DPPH with a value of 46.88 and the 100% SF at the 100 µg/ml had the least antioxidant activity with a value of 10.55%. The 80% extract also had the highest total phenolic content, and iron chelating activity at higher concentrations. While the highest saponin extraction yield was obtained by 100% fraction. The effect of 1mM Silver nitrate ($AgNO_3$) was evaluated for five minutes, and it was found to have stimulatory effect by remarkably increasing the antioxidant activities of each concentration tested from 86.08 to 65.29. The results from this study indicate that polyphenols could be better extracted using a correct mixture of acetone, methanol, ethanol and water because of polarity, and plant's saponin, being an effective antioxidant, could protect the body from oxidative damage by enhancing the antioxidant defence mechanism of the body system, and its antioxidant activity can be stimulated using silver nitrate or its nanoparticles.

Keywords: Saponin; Antioxidants; Silver nitrate; Aframomum melegueta; Differential fractions

INTRODUCTION

The aberrant generation of oxygen and nitrogen free radicals can cause severe damage to key cellular components resulting in cell apoptosis. However, antioxidants are capable of scavenging these free radicals, thereby altering their cytotoxic activities. Therefore antioxidants are the substances which protect cells from damage caused by unstable molecules known as free radicals. Antioxidant agents must then be of low cost and exhibit low side effects. As a result, plants are likely the best option for mankind as a natural source of antioxidants. Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing. However, antioxidant activity and extraction yield of plant not only depend on the extraction method, but also on the solvent used for extraction, therefore polar solvents are frequently used for recovering of polyphenols from plant matrices, the most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate.

Correspondence to: John Ajayi, Department of Biochemistry, Federal University of Technology, Akure, Nigeria, E-mail: ajayijohn23@gmail.com

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Alligator pepper, is a plant with the botanical name *Aframomum melegueta*, a widely used spice in several parts of the world, and it is also known as grains of paradise. Its indigenous names include Atare among the Yoruba, Ose-oji among the Ibo, and Citta among the Hausa. The seeds are rich in phytochemicals such as flavonoids, phenolic compounds, tannins, steroids, saponin, terpenoids, cardiac glycosides and alkaloids. The seeds have been known to improve sexual function in folkoric medicine. It is also used in West Africa as a remedy for variety of ailments such as stomach ache, snake bite, diarrhoea, and widely used in the treatment of hyperprolactinaemia. According to Gismondi, the extracts of the plant possess anti-proliferative and differentiative effects. In a work by, the extracts was demonstrated to possess therapeutic action against certain tested bacterial strains which suggests that it could be effective in the treatment of gastrointestinal infection, nausea, respiratory problems, cold, fever, allergies, urinary tract infection and fungi infections.

The saponin of the plant was chosen as a choice metabolite in the present work. Saponins are composed of one or more hydrophilic glycoside moieties along with a lipophilic and derived aglycone and finally, one or more chains. They are a large family of amphiphilic glycosides of steroids and triterpenes found in plants and some marine organisms. They possess antioxidant and anticancer properties, and most have surface active and cholesterol binding properties. Many saponins are good detergents and produce stable foams in water. This beneficial use was supported by the findings, who proposed that saponins lower the surface tension and possess emulsifying properties, they also tend to alter the permeability of the cell wall and therefore exert a general toxicity on all organized tissue. The haemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for membrane sterols, particularly cholesterol with which they form insoluble complexes. They can be easily extracted using water, ethanol, or methanol because of their solubility in them.

PREPARATION OF PLANTS SAPONIN EXTRACT

Total saponin was prepared according to the method of Fenwick and Oakenfull. The saponin fraction was then purified. Total saponin is usually prepared using acetone and methanol as extraction solvent in the ratio 10:1.

Three different fractions (80%, 90%, and 100%) of Alligator pepper saponin extracts was prepared, by the following steps;

1. A quantity of 4g of the powdered plant was dissolved in 20mls of acetone.

2. The solution was then allowed to stand for 24hrs.

3. The solution was then filtered using a clean white handkerchief.

4. The filtrate was then concentrated for about 24hrs.

5. A quantity 2mls of methanol was then added to the concentrate and then allowed to stand for about 24hrs.

6. The solution was then re-filtered and the filtrate was concentrated for about 24hrs.

The saponin concentrate from the various fractions of each plant was then weighed, and then each was separately dissolved in 10mls of 70% ethanol. Four different concentrations of alligator pepper was prepared from the stock solution by dilution.

PREPARATION OF DIFFERENT ALLIGATOR PEPPER SAPONIN FRACTIONS

To prepare 80% SF of alligator pepper;

- A quantity of 4g of the powdered plant was dissolved in 20mls of 80% acetone.
- The solution was then allowed to stand for 24 hrs.
- The solution was then filtered using a clean white handkerchief.
- The filtrate was then concentrated for about 24 hrs.
- A quantity of 2mls of 80% methanol was then added to the concentrate and then allowed to stand for about 24 hrs.

DISCUSSION

Antioxidant activity, significantly increases with the presence of high concentration of total phenol and flavonoid contents. The results obtained from the present work shows that the lowest extraction fraction (80%) at the 150µg/ml had the highest total polyphenolic content, with a value than the 90% and 100% extraction fraction, This could be because phytochemical content increases with increased concentration, and also because polyphenolic compounds, being hydrophilic are better extracted using a correct proportion of aqueous-organic phase rather than an organic phase alone which agrees with the findings of Do, who reported that the total phenolic content on using 75% aqueous methanol was higher than using 100% aqueous methanol of *Limnophila aromatica*. However, saponin yield increased with an increase in the extraction fraction. This suggests that saponins, because of their more hydrophobic nature are better extracted in an organic phase. This is in agreement with previous studies.It was observed that the antioxidant activities of the plant increased with lower extraction fraction rather than the higher fractions, as the results of the DPPH radical scavenging indicates that the 80% saponin fraction yielded an activity for the 90%fraction and for the 100% fraction all at a concentration of 150µg.ml. The observed increase in the DPPH radical scavenging activity of the saponin rich extract of the plant which reduced with increased extraction fraction of the organic solvents could be because hydrophilic phyto-constituents such as polyphenols are responsible for the plant's antioxidant activity rather than the hydrophobic phyto-constituents such as saponin, reported that extraction of the root of S. chinensis with absolute organic solvents or water was not effective as compared to a lower fraction of 50% acetone which demonstrated high antioxidant capacity. 80% acetone was also reported by Kamaludin and Jaafar as the best concentration of solvent to extract the antioxidant activity from flower of Senduduk herb with a value as compared to the 20%, 40%, 60% and 100%. According to Toudert, the 70% methanolic extracts of Ampelodesma mauritanica, had the highest DPPH and ABTS

radical scavenging activity at values of respectively for the leave extracts, and respectively for the root extracts, which was then followed by the 80% fraction, and the least was the 100% fraction. This also agrees with the findings of this work.

The effect of concentration on the bioactivities of plants was evaluated through the in vitro parameters and can be concluded that plants bioactivities are concentration dependent. i.e plants bioactivities increases at higher prepared concentrations of the plant. The in vitro Iron chelating activity of the plant's saponin was tested to evaluate the effect of concentration on bioactivity using the 80% fraction, and it was observed that the values significantly increased from 110.53 at a concentration of 50µg/mls to 191.05 at the150µg/mls concentration.

Chelating property of plants affords protection against oxidative damage and Iron overload. Iron, decomposes lipid peroxides into peroxyl and alkoxyl radicals which can initiate the propagation stage of lipid peroxidation. Adefegha and Oboh, also demonstrated that extracts from Aframomum melegueta was able to significantly decrease the brain MDA levels in rats, during an induced lipid peroxidation. This results suggest that the plant could be used in the treatment of oxidative stress associated diseases such as cancers, diabetes, neurodegenerative diseases etc.

The in vitro hydrogen peroxide scavenging activity revealed that both saponin fractions of the alligator pepper seed possessed striking hydrogen peroxide scavenging activity, and hence may be able to inhibit hydrogen peroxide linked oxidative processes in the body. However, the 85% SF at the 250µg/ml concentration produced a significantly higher ($p<0.0001$) in vitro hydrogen peroxide scavenging activity with a value of 440.76.

DPPH consists of stable free radical molecules and is readily reduced by accepting hydrogen or electron from nanoparticles. In the present work, Silver nitrate was found to significantly increase the DPPH radical scavenging activity of the plant saponin extract, and the % increase was found to be concentration dependent. Thus, the % increase of the silver nitrate was found to increase from 10.99% to 40.86% as the concentration increased from 50µg/ml to 150µg/ml. This is in agreement with the results of Mohan who opined that Silver nanoparticles was able to increase the antioxidant activity (DPPH radical scavenging activity) of *Canthium coromandelicum* extract, and that this activity increases with the increase in concentration of the plant extract and the silver nanoparticles. Thus we can deduce that silver nitrate and silver nanoparticles synthesized from plant extract has higher antioxidant activity than the plant extracts themselves, possibly because of their large surface area and hence could be a good antioxidant boost. This research revealed that the DPPH radical scavenging activity of silver nitrate on the saponin extract of the *Aframomum melegueta* plant at the 50µg/ml concentration was 95.54% and was found to be higher than that of *Canthium coromandelicum* which was about 80% at 60µg/ml concentration as was reported by Mohan, has also reported the DPPH radical scavenging activity of silver and gold nanoparticles synthesized using aquoues extract of Angelica pubescens Maxim.

CONCLUSION

The results obtained from the present study suggest that the fraction of the extraction solvents and the concentration of the plant extracts contribute to the antioxidant activity of any given plant.

From the present work, and the results stated from other researchers, we can safely conclude that acetone and methanol at about 70% or 80% fraction could be better extraction solvents for the antioxidant activities of phenolic compounds, but better still, a combination of the two solvents in the appropriate ratio as seen in this research could even prove more effective. Moreover, saponin extracts from this plant could protect the body from oxidative damage by the mechanisms of iron chelation, and hydrogen peroxide scavenging, thus suggesting them to be cheaper and more readily available alternatives to the conventional drugs in the treatment of oxidative stress associated diseases such as cancers, diabetes, cardiovascular diseases, neurodegenerative diseases etc.

Finally, Silver nitrate could also be a good compliment to the antioxidant activities of plant.

It is recommended that further researches should be carried out in vivo to elucidate non-toxic concentrations of silver nitrate that can complement the antioxidant activities of plants.

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