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SCAN ME

Research Paper

ASSESSMENT OF SYNERGY BETWEEN ANTIOXIDANT POTENTIALS OF *Alpina calcarata* LEAF EXTRACT AND ANTIOXIDANT ENZYMES ACTIVITY

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Abstract

The present research was conducted to assess the synergy between antioxidant potentials of *Alpina calcarata* leaf extract and its effect on antioxidant enzymes activity. Extracts were prepared from the leaf of the plant with aqueous and ethanolic solvents system at 5.0% concentration. Antioxidant inhibitory potential of Alpina calcarata leaf extract against Pb^{2+}-induced oxidative stress was investigated in liver and kidney tissues of albino rats. The effect of the extract on activity of selected defensive antioxidant enzymes; reduced glutathione (GSH) and glutathione peroxidase (GPx) was also assessed. The results obtained indicate the potency of the extract against oxidative stress with 89.8% inhibitory potential at maximum extract concentration (160mg/ml) in the liver which was higher than (75.2%) obtained in kidney tissue. Besides, the interaction of the extract with the activities of GSH and GPx antioxidant enzymes was synergistic where high level of enzyme activity was concomitant with increase extract concentration. Hence, oxidative stress in in liver and kidney tissues could be potentially managed or prevented by dietary intake of Alpina calcarata without posing adverse effect on inherent antioxidant enzymes' activities.

Key words: Oxidative stress, liver, kidney, antioxidant-enzymes, lead.

INTRODUCTION

Several studies on phytomedicines have reported that phenolic compounds protect against oxidative stress [1]. Some of these medicinal plants have been investigated for their antioxidant properties and used for the treatment of various infections [2]. Most of

the bioactive metabolites from these plants especially flavonoids demonstrated potent antioxidant activity in vitro and in vivo [3]. Many synthetic antioxidants and metal chelator components have also exhibited toxic or mutagenic effect coupled with suppression of body immunity which have shifted attention towards naturally occuring antioxidants [4]. *Alpina calcarata* is grown specifically for its essential oils in its leaves and barks where thymol, eugenol, citral, geraniol and linalool as well as high level of selenium have been extracted [5]. Medicinal plants play pivotal role in the health care delivery services in both ancient and mordern medicine. Indians and Chinese system of medicine depends solely on plant based drugs to treat various human ailments since they contain different components of therapeutic value [6]. Besides, plant based drugs still relevant and remain very important source of therapeutic agents due to availability, relatively cheaper cost as well as non-toxic nature compared to unorthodox medicine [7]. Most medicinal plants contain antioxidant compounds that protect the cells against the damaging effects of reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide [8]. These radicals are generated in human body via aerobic respiration or rather from exogenous sources and thus play crucial roles in the development of various ailments such as arthritis, asthma, cardiovascular disorders, neurodegenerative and parkinson diseases [9]. However, phenolic compounds from medicinal plants possess strong antioxidant activity and may help protect the cells against oxidative assault caused by these free radicals [10]. Hence, this study was designed to investigate and evaluate the antioxidant potential of *Alpina calcarata* leaf extract and its effect on defensive antioxidant enzymes.

MATERIALS AND METHODS

Collection of Plant Sample

Fresh leaves of *Alpina calcarata* plant were fetched from a reserved virgin forest near Ikare-Akoko township, Ondo-State, Nigeria. The plant was authenticated at the herbarium centre of Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Preparation of Plant Extracts

The aqueous extract was prepared by extracting 150g of powdered sample in cold sterile distilled water, aggitated with mechanical shaker, and filtered via buchner funnel with No 1 whatman's filter paper, frozen at -40^{0}C and dried with freeze dryer for 72hrs.

The percentage yield of 11.33% was obtained [11] for aqueous extract while 120g each of powdered sample was extracted with (70%) ethanol respectively.. The mixture was decanted and filtered with No 1 Whatman's filter paper which measured up to 600mls and was evaporated to dryness to give 9.96% yield.

Chemical Reagent

Chemicals and reagents used such as leadacetate, gallic acid, thiobarbituric acid (TBA), Tris-HCl buffer, sodium dodelcylsulphate, acetic acid, TCA were sourced from Sigma-Aldrich, Inc., (Steinheim, Germany) and were of analytical grade while the water was glass distilled.

Lipid Peroxidation Assay

Lipid peroxidation / TBARS assay was conducted using the modified method of Ohkawa et al., [12]. Tissue homogenate was prepared from decapitated rats under mild diethyl ether anaesthesia and the liver and kidney were excised and subsequently homogenized in cold saline (1/10 w/v) in Teflon glass homogenizer. The homogenate was centrifuged for 10min at 3000 xg to yield supernatant that was used for lipid peroxidation assay.

Antioxidant Enzymes Assay

The methods of Beutler et al., [13] and Rotruck et al., [14] were used to assess the effect of the extract on GSH and GPx activities respectively. The chromophoric products from reaction of Ellman's reagent with reduced glutathione, 2-nitro-5-thiobenzoic acid with characteristic absorbance wavelengh taken at 412nm.

RESULTS

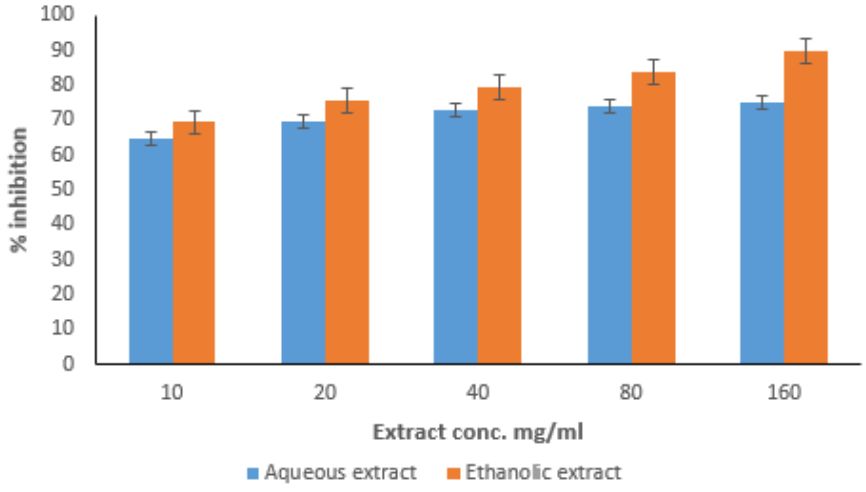

Fig.1: Inhibitory potential of *Alpina calcarata* extracts against Pb^{2+}-induced lipid peroxidation in rat liver

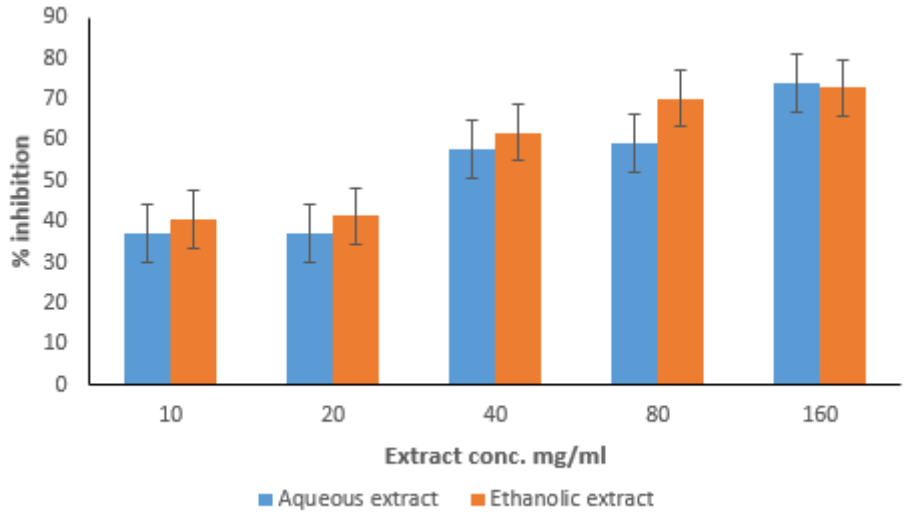

Fig.2: Inhibitory potential of *Alpina calcarata* extracts against Pb^{2+}-induced lipid peroxidation in rat kidney

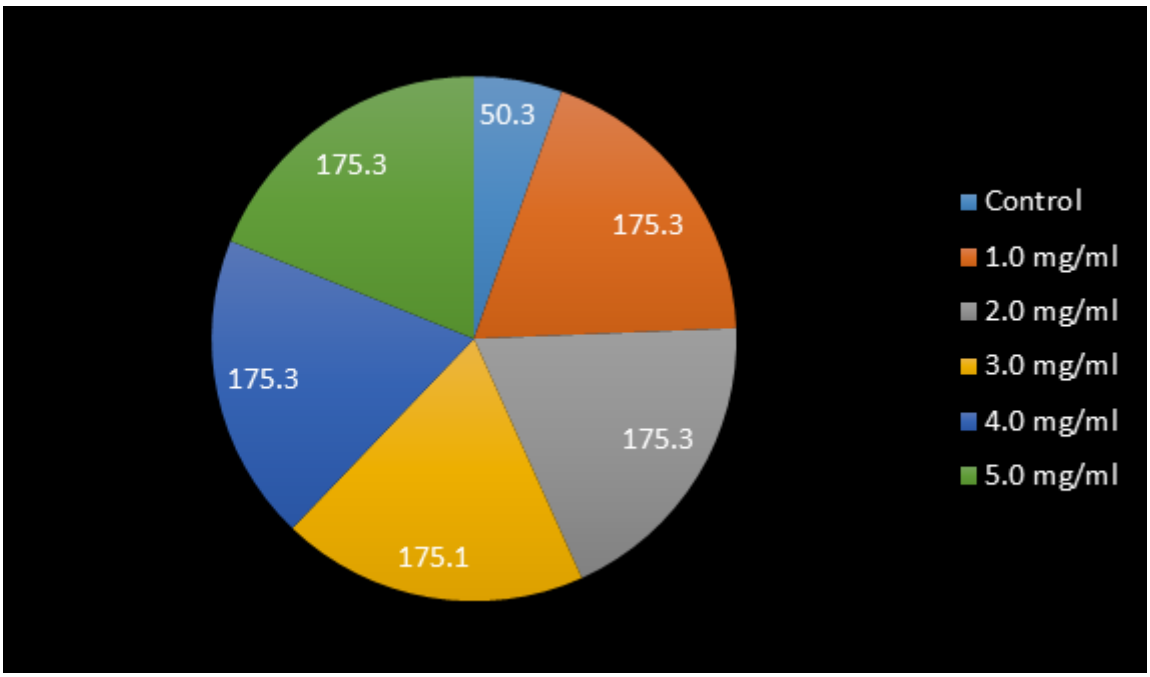

Fig.3: Effect of *Alpina calcarata* aqueous extract on glutathione enzyme activity

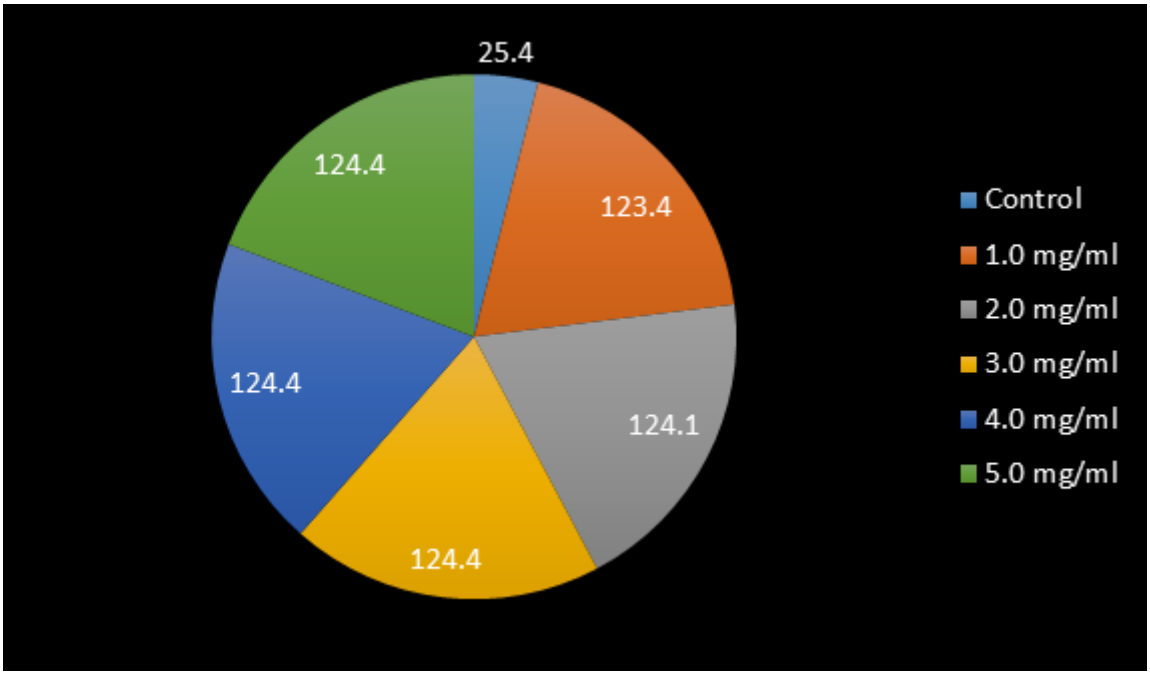

Fig.4: Effect of *Alpina calcarata* aqueous extract on glutathione peroxidase activity

DISCUSSION

Free radicals are constantly generated resulting in extensive damage to tissues and macromolecules leading to development of various diseases. Medicinal plants are employed as alternative therapy to mitigate against the oxidative stress related diseases [15]. Results from this study showed that *Alpina calcarata* extract demonstrated high inhibitory potential (89.8%) against Pb^{2+}-induced lipid peroxidation in liver tissue which was higher than that obtained from kidney tissue (75.2%). Pb^{2+} can stimulate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and hydroxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (16). Besides, the inhibitory potential of the extract might be due to its reducing power and hydrogen donating ability which accounts for its antioxidant activity of phenolic compounds (17). The results show that ethanolic extract of *Alpina calcarata* demonstrated higher inhibitory potential than aqueous. The effect of the extract on GSH and GPx activities was synergistic with concomitant increase in their activities corresponding to increase in extract concentration. This could be attributed to the presence of selenium in the extract which generally activate defensive antioxidant enzymes to prevent the rise in concentration of free radicals [18]. The presence of selenium in the plant extract is perhaps responsible for activation of GPx activity where

it converts H_2O_2 into water [19] and thus, prevent membranes of the animal tissues from attack by H_2O_2 and stop propagation of free radicals which generally result in oxidative stress [20].

CONCLUSION

It can be inferred from the results above that *Alpina calcarata* plant leaf possesses high antioxidant potentials and its dietary intake is safe and synergistic with defensive antioxidant enzymes activities.

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DECLOSURE OF CONFLICT OF INTEREST

No conflict of interest

REFERENCES

[1] Proestos, C., Boziaris, I.S., Nychas, G.J., Komaitis, M. (2006). Analysis of flavonoids and phenolics in aromatic plants. Investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 95: 664-667.

[2] Gyamfi, M.A., Yonamine, M., Aniya, Y. (1999). Free radical scavenging action of herbs from Ghana; Thonningia sanguine on experimentally induced liver injuries. General Pharmacology, 32: 661-667.

[3] Chouni, A. and Paul, S.(2018). A review on phytochemical and pharmacological potential of Alpina calcarata. Pharmacogn. Journals 10: 9-15.

[4] Roja, G. and Rao, P.S. (2000). Anticancer compounds from tissue culture of medicinal plants. J. Herbs-spices Medicinal Plants, 7: 71-102.

[5] Sulistiarini, D., Oyen, L.P.A., Nguyen, X.D. (1999). Extraction methods of essential oils of Ocimum gratissimum in South-East Asia. Prosen Foundation, Bogor, Indonesia, pp. 140-142. [6] WHO, (1993). Research guildlines for evaluating safety and efficacy of herbal medicines. Manila World Health organization regional office for western pacific, Switzerland.

[7] Sofidiya, M.O., Odukoya, O.B., Familoni, S.T., Inya-Agha. (2006). Free radical scavenging activity of some Nigerian medicinal plant extracts. Pakistan Journal of Biology and Science. 9: 1438-1441.

[8] Miron, D.M., Crestani, R.M., Schetinger, M.V., Morsch, B. (2005). Effect of herbicide clomazone, quinclorac and metsulfuron-methyl on acetylcholinesterase activity in silver catfish. Ecotoxicol. Saf. 61: 398-403.

[9] Aghor, A.G. and Ngogang, Y.J. (2005). Toxicity ofherbal preparations. Cam. J.Ethnobotany 101: 23-28

[10] Weisburger, J.H. (1999). Mechanism of action of antioxidants as exemplified in vegetables, tomatoes and teas. Food Chem. Toxicol. 37 (9-10): 943-948.

[11] Aguawa, C.N. and Mittal, G.C. (2009). Study of antiulcer activity of aqueous extract of Pyrenacanthia stautii leaf using various model of experiment for gastric ulcer in rats. Eur. J. Pharmacol. 74: 215-220.

[12] Okhawa, H., Ohishi, N. Yagi, K. (2009). Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358.

[13] Butler, J., Gyamfi, M.A., Yonamine, M., Aaniya, Y. (1993). Estimation of glutathione in the liver and brain supernatants. General Pharmacology. 32: 661-667

[14] Rotruck, N.H., Hertwig, B., Streb, J.(1993). Determination of glutathione peroxidase activity. General Pharmacology. 32: 551-555.

[15] Usoh, I.F., Akpan, E.J., Etim, E.O., Farombi, E.O. (2005). Antioxidant action of dried flower extract of Habiscus sabdariffa.. L on sodium arsenite induced oxidative stress in rats. Pakistan journal of Nutrition , 4: 135-141.

[16] Halliwell, B. and Gutteridge, J.M.C. (1990). Roles of free radicals and catalytic metal ions in human diseases. Enzymol. 186: 18-85.

[17] Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47: 3954-3962.

[18] Ediziri, H., Ammar, S., Souad, L., Mighiri, Z. (2012). In vitro evaluation of antioxidant enzymes activity in rat liver. Enzymol. 78: 252-256.

[19] Chiu, D., Vichinsky, E., Yee, M., Kleman, K., Lubin, B. (2002). Glutathione peroxidase, Vitamin E and sickle cell anemia. Annals Acad. Sci. 393: 323-335.

[20] Das, S.K. and Nair, R.C. (2000). Defensive antioxidants activities and lipid peroxidation of normal and sickled erythrocytes. Br. J. Hematol. 44: 87-92.