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The Effect of *Sacoglottis gabonensis* and its Isolate Bergenin on Doxorubicin - Ferric Ions (Fe^{3+}) - Induced Degradation of Deoxyribose

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The research work was conducted to investigate the effect of *Sacoglottis gabonesis* (*S. gabonensis*) bark extract and one of its phytochemical constituents bergenin, on the degradation of deoxyribose side effect of the anticancer drug, doxorubicin were investigated *in vitro*, in comparison with vitamin E (d - ∝ - tocopherol) and 4% ethanol (C_2H_5OH) control, respectively. The reaction was monitored as TBA activity detected spectrophotometrically at 532nm. Treatment with *S. gabonensis* bark extract bergenin and vitamin E inhibited doxorubicin - Fe^{3+}- induced degradation of deoxyribose of the oxidative degradation of deoxyribose appears to be inhibition of the propagation of lipid peroxidation.

Key words: *Sacoglottis gabonensis*, bergenin, vitamin E, doxorubicin, deoxyribose, lipid peroxidation, antioxidants

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Introduction

Sacoglottis gabonensis Urban (Family Humiriaceae) is a tropical rain forest tree. Its stem bark is used as an additive in the treatment of raphia palmwine, an alcoholic beverage in Southern Nigeria especially among the rural communities of Abia, Imo, Akwa Ibom, Cross Rivers, Rivers and Delta States. It is used in traditional medicine practice, in rescucitating pregnant mothers, after delivery and for treatment of microbial diseases. Research done in this and other laboratories suggested that certain constituents of the aqueous extracts of the bark posses antioxidant properties (Madusolumuo, 1993; Ekong and Ejike, 1974) and may influence biological responses to foreign compounds like hepatocarcinogen, aflatoxin B_1, (Okoye and Neal, 1988a; 1988b; 1991). Madusolumuo (1993) investigated the effect of bark extract on the anticoagulant side effects of two foreign compounds, acetaminophen and acetylsalicylic acid. The extract has also been shown to exert significant influence on the metabolism and disposition of aflatoxin B_1 and induction of neoplastic tissues as determined by γ glutamyl transpeptidase activity (Okoye and Neal, 1988a; 1988b; 1991).

S. gabonensis bark extract has been reported to exhibit antioxidant properties against oxidants induced lipid per oxidations. For instance, it was reported to spare the tissue depletion of hematological parameters (red blood cells, packed cell volume) and inhibited the proliferation of white blood cells (total & differential) during lipid peroxidation/oxidative stress by phenylhydrazine (Maduka *et al.*, 1999a) *in vivo*, Similarly, the bark extract inhibited typical free radical reactions *in vitro* redox systems involving oxidants paraquat (Maduka *et al.*, 1999a) and carbon tetrachloride (Maduka, 1999b)

Bergenin, an isolate of *S. gabonensis* was also reported to inhibit the *in vitro* free radical driven lysis of mammalian erythrocytes by peroxyl radicals of carbon tetrachloride (CCl_4) by inhibiting the propagation of lipid peroxidation pathway *in vitro* (Maduka and Okoye, 2000). Both paraquat and CCl_4 cause free radical-induced damages *in vivo* (Bus *et al.*, 1974; Sugiyama *et al.*, 1993) by similar mechanism with doxorubicin, an anticancer drug known to induced peroxidation of cardiac lipids (Gutteridge, 1984).

Therefore, the research work was conducted to investigate the free radical reaction of doxorubicin -Fe^{3+} -induced oxidative degradation deoxyribose in the presence of bark extract, bergenin and vitamin E, an antioxidant of known action used usually in the determination of biological antioxidant properties (Nutrition Reviews, 1988). The objective of this work was to ascertain if *S. gabonensis* stem bark extract and bergenin have antioxidant properties and if so, determine if bergenin is a major constituent of the bark responsible for the observed antioxidant action. Another objective of the work was to deduce the mechanism of inhibition by *S. gabonensis* and bergenin.

Materials and Methods

Freshly harvested samples of *S. gabonensis* stem bark were purchased from Ekeapara market in Ngwa, Aba, Abia State of Nigeria and refrigerated. The 1:10 (bark weight volume) aqueous ethanol extract (C_2H_5OH) of *S. gabonensis* was prepared as described by Madusolumuo (1993); Okoya and Neal (1991). Doxorubicin hydrochloride was obtained from Erba, Italy and vitamin E (d ∝- tocopherol) from Ferrosans Ltd, England. All other reagents (2-deoxy-D-ribose, ferric chloride ($FeCl_3$), sodium hydroxide (NaOH) and sodium chloride (NaCl) were of analytical grade and obtained from BDH, Poole Dorset, UK. Pure bergenin powder were extract by the procedure of Madusolumno (1993).

The degradation of deoxyribose as thiobarbituric acid (TBA) reactive material it was determined essentially by the method of Gutteridge (1984). The doxorubicin in the presence of ferric ions (Fe^{3+}) forms a complex can activate dioxygen to generate reactive radicals capable of degrading deoxyribose as well as form a species greatly stimulatory towards lipid peroxidation. The reaction can be monitored as TBA detected spectrophotometrically at 532nm. The TBA reactivity is based on the reaction of carbonyl with 2-thiobarbituric acid during lipid peroxidation. Antioxidants inhibit the degradation of deoxyribose. The only modification of the original method of Gutteridge (1984) was that due to technical problems in the sizes of cuvette, 2.0ml of phosphate buffered saline (PBS), pH 7.4 instead of 0.2ml was used as the incubation medium. 0.2ml of liver homogenate prepared by the method of Maduka (2000), the antioxidants (1ml of 1 M d- ∝ - tocopherol, 1ml of 4% C_2H_5OH aqueous 1:10 (w/v) stem bark extract and 4% C_2H_5OH control were added to the final reaction mixtures, respectively before the addition of 0.1ml of 1mM $FeCl_3$ source for membrane lipid. After incubation for 1, 2 and 3 hrs in three sets of tubes at 37°C, 0.5ml of 1% Na-2-thiobarbituric acid reagent in reaction-buffer was added to each of the tubes followed by 0.5ml of 2.8% (w/v) trichloroacetic acid solution (TCA). All the tubes were mixed, heated for 15 minutes at 100°C, cooled and their absorbance were used to evaluated degradation of deoxyribose as TBA-reactivity. Similarly, several solvent controls were setup and run like the tests. Tubes A served as the overall control, B as Fe^{3+} blank, C as d-∝-tocopherol, D as *S. gabonensis* test, E as deoxyribose blank, F as doxorubicin blank while G served as the 4% C_2H_5OH control. Statistical analysis was carried by the student t-test.

Results and Discussion

The results are presented in Fig. 1 as thiobarbitric acid reactivity (TBA reactivity), a reliable though non-specific Index of lipid peroxidation. Bergenin, *S. gabonensis* bark extract and d-∝- tocopherol inhibited doxorubicin-Fe^{3+} induced fragmentation of deoxyribose as shown by decreases in TBA values when compared with non-antioxidant controls. Under the same experimental conditions, bergenin was the best inhibitor (antioxidant) followed by *S. gabonensis* and then ∝- tocopherol and 1ml of 4% ethanol of (1:10 w/v). *S. gabonensis* stem bark extract equlavlent of 100mg dry powder are used as antioxidant tests respectively). Doxorubicin, Fe^{3+} and deoxyribose general control) yielded the highest TBA values confirming that deoxyribose was degraded to yield carbonyl compounds which were detected spectrophotometrically at 532nm.

However, the reaction profile showed that the three antioxidants: bergenin, *S. gabonensis* bark extract and vitamin E inhibited the reaction over the 3 hr period of incubation. The reaction profile also showed that propagation was being inhibited and retarded as the reaction progressed. At time 1 hr, there was no statistical differences between the bark extract in 4% C_2H_5OH and doxorubicin and Fe^{3+} (tubes G and B, respectively) blanks.

Data has been presented to show that *S. gabonensis* stem extract and bergenin, inhibited the production of lipid hydroperoxyl aldehydes as determined by malonaldehyde (TBA reactivity) formation in the degradation of deoxyribose over a long period of three hours by doxorubicin - Fe^{3+} complex. In this *in vitro* free radical mediated reaction system, the degraded materials were detected non-specifically as TBA reactive materials at 532nm.

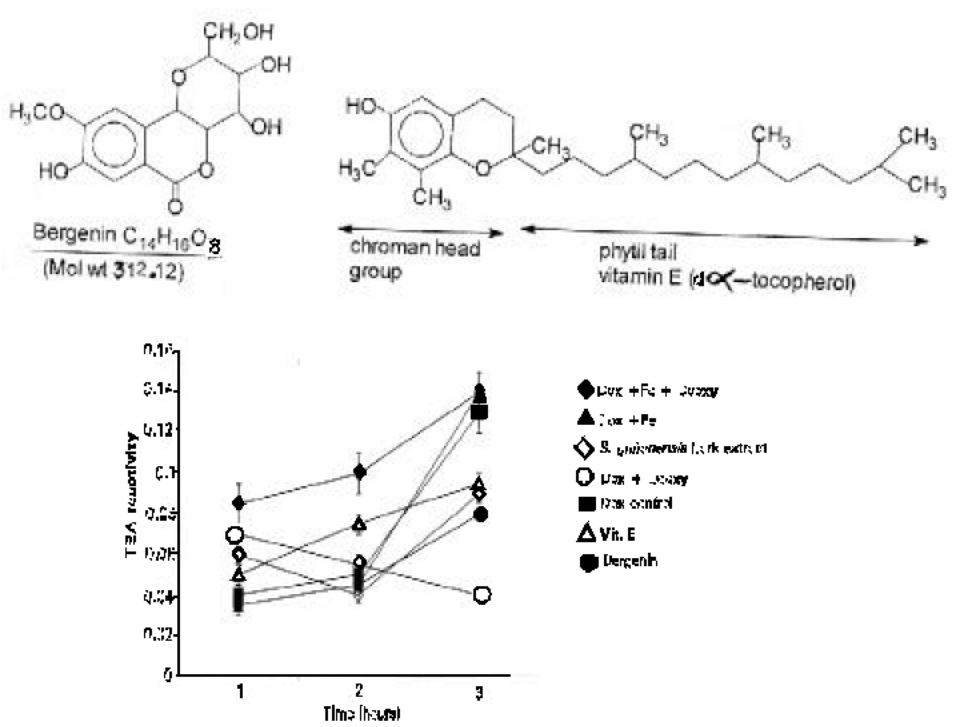

Fig. 1: The inhibition of degradation of deoxyribose by *S. gabonensis*, vit. E. and bergenin as TBA reactivity at 532nm.

The inhibition of degradation of deoxyribose by the *S. gabonensis* aqueous stem bark extract and bergenin as evidenced by comparatively reduced TBA values compared favorably with lipid peroxidation protecting agents like vitamin E.

The results obtained suggested that both bergenin and the bark extract acted as antioxidants by reducing the rate of lipid peroxidation product, lipid hydroperoxide aldehydes just like vitamin E, a known antioxidant used in the assessment of biological antioxidant properties (Nutrition Reviews, 1988). Vitamin E values correlate or are directly related to assessment of lipid peroxidation levels with other indices of biological oxidation (Dilliard and Litov, 1977; Singh and Singh, 1982; Stoyanovsky *et al*., 1995). At simlar molar concentrations and under the same experimental conditions, bergenin acted as a stronger inhibitor of degradation of deoxyribose than crude extract followed by vitamin E.

TBA reactivity as mentioned earlier is reliable, though, non-specific index of assessment of lipid peroxidation and attack on carbonyl (Gutteridge, 1984; Wills, 1987). In the original design of the doxorubicin -F^{3+} -induced free radical degradation of deoxyribose, doxorubicin-ferric complex forms ferrous species which activate dioxygen (O_2) to radicals (2O•) which were said to degrade deoxyribose and cause lipid peroxidation. This investigation suggested that the mammalian toxicity of doxorubicin, an anthracycline anti-tumor antibiotic (Gutteridge, 1984; Gilman *et al*., 1985) may be a consequence of lipid peroxidation. Also the experimental results have presented evidence that the extract and bergenin inhibited and reduced the rate of propagation of the free radicals fragmenting the deoxyribose and later detected as fatty acid carbonyl compounds. This trend of results is similar to the Maduka *et al*. (1999b) report that the crude extract and bergenin inhibited the *in vitro* reduction of paraquat (PQ) to free radical ($PQ^{\bullet}$) by inhibiting propagation (Maduka *et al*., 1999a) and our later reports that both antioxidants acted as antioxidant protectors of mammalian erythrocytes against lysis by peroxyl radicals of CCl_4 (Maduka *et al*., 1999b). These results suggested that bergenin is a major component responsible for the observed antioxidant action of *S. gabonensis* stem bark extract.

The ultimate peroxidation stimulating principle is a doxorubicin Fe^{3+} complex (Gutteridge, 1984). The iron of doxorubicin Fe^{3+} complex undergoes reduction to Fe^{2+} which is believed to activate molecular oxygen (O_2) to reactive radicals (2O•) responsible for the peroxidation of membrane lipids, degradation of deoxyribose and attack on nitrogenous moieties of the nucleic acids (Gutteridge, 1984). This would suggest that the biological activity of doxorubicin and its side effects, especially those depen dent on its pro-oxidation effect, may be altered in the presence of antioxidants. For instance, if antioxidants are implicated in inhibiting the toxic side effects. The inhibition of the doxorubicin -Fe^{3+} - induced free radical reactions by the bark extract, bergenin and vitamin E suggests that they could interfere with doxorubicin therapy or affect metabolic response to doxorubicin. The ability of bergenin and bark extract, to inhibit some of the toxic side effects like degradation of deoxyribose and inhibition of membrane lipid peroxidtion, could enhance and popularize the use of doxorubicin in the treatment and management of cancer.

Through the mechanism of inhibition of doxorubicin -Fe^{3+} induced degradation of deoxyribose in not yet known, it is clear that the antioxidants inhibited the reactive species responsible for the release of TBA reaction product from

deoxyribose as clearly shown by significantly reduced values of lipid hydroperoxide aldehyde (malonaldehyde). The results have also shown that propagation was being inhibited since malonaldehyde is a product of disintegration of lipid hydroperoxide which is fundamental step in propagation of the lipid peroxidation pathway (Dilliard and Litov 1977; Dilliard *et al.*, 1977; Dumelia and Tappel, 1977). The mechanism of *S. gabonesis* and bergenin inhibition and antioxidant action were probably enhanced by the amphipathic structural arrangement of bergenin due to the presence of polar and lipid substituents in bergenin, as isocoumarin. (Sugiyama *et al.*, 1993) has reported that the amphathic nature of purpurogallin was responsible for the inhibition of lysis of erythrocytes by peroxyl radicals. Also (Burton and Ingold, 1984) ascribed the antioxidant deactivating properties of vitamin E (d-∝-tocopherol) to its structural arrangement involving the heterocyclic ring of the chroman head group, which plays a very important role in optimizing antioxidant activity.

Through there is no stated value of the amount of *S. gabonensis* stem bark used as an additive in palmwine in Southern Nigeria, the quantity used depends on the taper consumer response. Okoye and Neal (1988a) established the minimum dilution at which absorption in the 510-400 region was distinguishable from the base line. Also the UV spectrum (520-200nm) of the extract showed a major absorption peak at 270nm. Whether the doses are relevant to *in vivo* concentration will be established and extrapolated in subsequent research though results so far tend to support that they are. The results of this investigation have encouraged us to conclude that both *S. gabonensis* stem bark extract and bergenin possess antioxidant properties against oxidative degradation and hence, lipid peroxidation, *in vitro*. It is also being concluded that the mechanism of the observed action appears to be by inhibition of propagation of lipid peroxidation.

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