Effectiveness of 3 Polyherbal Formulations (EcXaPu, EcXa, and EcPu) on the Management of Oxidative Stress and Hyperglycemia

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ABSTRACT: Oxidative stress and hyperglycemia are major disorders involved in the occurrence and severity not only of chronic non-communicable diseases but also of infectious pathologies. This study aimed to evaluate the in vitro antioxidant and antihyperglycemic properties of EcXaPu, EcXa, and EcPu. The antioxidant properties were evaluated using 3 mechanisms: radical scavenging; reducing property, and metal chelating. Finally, the antihyperglycemic properties were evaluated by 2 mechanisms: glucose adsorption and cellular glucose capture. The different formulations showed their ability to scavenge DPPH, ABTS, and NO radicals with SC_{50} ranging from 2.75 to 3.51 mg/ml, from 2.6 to 2.76 mg/ml, and from 2.59 to 3.3 mg/ml, respectively. All the formulations also reduced MoO_4^{2+} and Fe^{3+} and chelated Cu^{2+} and Fe^{2+}. The different formulations adsorbed the glucose with glucose adsorption rates ranging from 72.83% to 87.01%. The different formulations also stimulated cellular glucose uptake, with uptake rates ranging from 31.9% to 50.71% in yeast cells and from 21.81% to 39.45% in muscle cells. These formulations could be potential agents to prevent and/or protect against biological disorders associated with oxidative stress and hyperglycemia.

KEYWORDS: Polyherbal formulations: EcXaPu, EcXa and EcPu, oxidative stress, hyperglycemia

Introduction

The first cause of death in the world are the chronic non-communicable diseases, which accounting for 71% of deaths and killing an estimated 41 million people each year.[1] Many metabolic risk factors are associated with these diseases, including oxidative stress and hyperglycemia, which are also responsible for the severity of these diseases.[2] The relationship between stress and hyperglycemia has also been demonstrated, as one can cause and/or aggravate the other and vice versa. Thus, hyperglycemia through numerous pathways such as activation of protein kinase C, the polyol, and hexosamine pathway, production of advanced glycation end products, and mitochondrial oxidative phosphorylation can generate a state of oxidative stress.[3] On the other hand, these 2 disorders are also associated with the occurrence of complications of infectious diseases.[4,5] Numerous studies have shown the involvement of both oxidative stress and hyperglycemia in the severity of the global pandemic SARS CoV-2 infection.[6-8] In view of the strong involvement of oxidative stress and hyperglycemia in the occurrence of both chronic non-communicable diseases and infectious diseases, the management of these 2 disorders is essential and remains a public health challenge.

Numerous studies already showed the antioxidant and antihyperglycemiant properties of many plants extract from the Cameroonian pharmacopeia.[9,10] These properties are most often attributed to the presence in these plants of secondary metabolites in these plants, such as polyphenols, which are known for their multiple biological properties.[11,12] Herbal medicine is becoming more and more important with the diversity of natural resources and their abundance of bioactive compounds. Always in search of much more active substances, the combination of several plant extracts has long emerged in Asia.[13] However, it is only recently that this concept has been the subject of scientific publications in Cameroon. Indeed, in most traditional systems, non-communicable diseases such as diabetes are better managed by a combination of plants that can act through different mechanisms, than by individual plants.[14] According to a systematic review conducted in 2015, over the span of that year, work based on herbal formulations was mostly carried out on diabetes and oxidative stress.[15] Numerous antihyperglycaemic and antioxidant formulations have already been identified in India, such as the Mehani formulation[16] and ADPHF6.[17] In Cameroon, a study was also conducted on a plant formulation that is both an antioxidant and antihyperglycemiant.[18] Based on this observation, we were interested in a formulation based on the bark extracts of 3 plants from the Cameroonian pharmacopeia known for their antihyperglycaemic and/or antioxidant properties: *Enantia chloranta* stem bark,[19] *Xylopia aethiopica* fruit,[20] and *Piper umbellatum* leaves.[21] We aimed to evaluate the antioxidant and

antihyperglycemiant properties of the polyherbal formulations based on these 3 plants.

Material and Methods

Plant materials

Enantia chlorata stem bark, *Xylopia aethiopica* fruit, and *Piper umbellatum* leaves were harvested in January 2019 at Ondodo (East-Cameroon), January 2019 at Bafoussam (West-Cameroon), and February 2019 at Bazou (West-Cameroon) respectively. They were isolated, cut into small pieces and then dried in the sun till a constant weight was attained. After grounding, a powder was obtained.

Preparation of extracts. A mass of 100 g of each powder was mace red in 400 ml of mixture of water/ethanol (95%) in a ratio of 1:1 (v/v). After 48 hours of maceration, the extracts were filtered using Whatman #2 filter paper and concentrated by a rotavapor before drying in an oven at 50°C.

Preparation of herbal formulations. The polyherbal formulations were prepared as follows:

- F1 (EcXaPu): *Enantia chloranta* stem bark + *Xylopia aethiopica* fruit + *Piper umbellatum* leaves (1:1:1 w/w);
- F2 (EcXa): *Enantia chloranta* stem bark + *Xylopia aethiopica* fruit (1:1 w/w);
- F3 (EcPu): *Enantia chloranta* stem bark + *Piper umbellatum* leaves (1:1 w/w).

Quantitative determination of polyphenolic compounds

Determination of total polyphenol content. The polyphenol content was evaluated using the method described by Singleton and Rossi and Rossi.[22] To 30 μl of the formulation, 1 ml of Folin Ciocalteu was added. Thirty minutes after the incubation at 25°C, the absorbance was read at 750 nm using a spectrophotometer. Catechin was used as standard. The total polyphenol was expressed in microgram equivalence of gallic acid/g of the formulation.

Determination of flavonoid content. The flavonoid content was evaluated using the method described by Aiyegoro and Okoh.[23] A volume of 1 ml of formulation (1 mg/ml prepared in an ethanol solution) was added to 1 ml of aluminum chloride, 1 ml of potassium acetate, and 5.6 ml of distilled water. The mixture was incubated at 25°C for 30 minutes. The absorbance of the reaction mixture was read at 420 nm with a spectrophotometer. Quercetin was used as the standard. The flavonoid content was expressed in milligram equivalence of catechin/g of formulation.

Antioxidant properties

Scavenging radicals

DPPH assay. The DPPH scavenging properties of the formulations was evaluated by the method of Katalinić et al.[24]

In total, 0.975 ml of a methanolic solution of DPPH (0.3 mM) was added to 25 μl of formulations (1; 2; 3; 4; and 5 mg/ml). The mixture was kept in the dark and incubated at 25°C for 30 minutes. The DPPH methanolic solution without the formulations was used as a control. The absorbance of the mixture was read at 517 nm.

ABTS assay. The $ABTS^+$ scavenging properties of the formulations was evaluated by the method of Re et al.[25] The $ABTS^+$ solution (8 mM of ABTS, 3 mM of potassium persulfate in 25 ml of distilled water) was conserved at 25°C for 16 hours in the darkness. After 1:10 dilution of the $ABTS^+$ solution with ethanol (95%), a volume of 0.5 ml was added to 10 μl of formulations (1; 2; 3; 4; and 5 mg/ml). The mixture was incubated for 30 minutes at 25°C. The $ABTS^+$ solution without the formulations was used as a control. The absorbance of the mixture was read at 734 nm.

NO assay. The NO scavenging properties of the formulations was evaluated by Griess' reaction.[26] A volume of 2 ml of sodium nitroprusside (10 mM) dissolved in 0.5 ml phosphate buffer saline (0.025 M; pH 7.4) is mixed with 0.5 ml of different formulation (1; 2; 3; 4; and 5 mg/ml). The mixture was incubated at 25°C for 150 minutes. Then, 0.5 ml of incubation mixture was diluted with 0.5 ml of Griess' reagent (1% sulphanilamide, 2% Ophosphoric acid, and 0.1% napthyl ethylenediamine dihydrochloride). The control was done without the formulations. The absorbance of the mixture was read at 546 nm.

For each antiradical assay, the scavenging percentages were calculated as follows:

$$\text{Scavenging effect } (\%) = \left[(DOcontrol - DOsample) / DOcontrol \right] \times 100$$

The scavenging Concentration (SC_{50}) parameter was used for the interpretation of the results.[27]

Reducing property. This property was evaluated by the capacity of the formulations to reduce the MoO_4^{2-} and Fe^{3+}.

Phosphomolybdenum method (total antioxidant capacity). Total antioxidant capacity was measured by the method of Prieto et al.[28] A volume of 0.1 ml of formulations (5; 6.25; 7.5; 8.75; and 10 mg/ml) was mixed to 1 ml of reagent work (sulfuric acid [0.6 M], sodium phosphate [28 mM], and ammonium molybdate [4 mM]). The mixture was incubated at 95°C for 90 minutes. After cooling, the absorbance was read at 695 nm. The total antioxidant capacity was expressed in mg Equivalence of Trolox/mg of the formulation.

Reducing power method. The reducing power method was evaluated by the method of Oyaizu.[29] In total 0.5 ml of the formulation was added to 0.5 ml of sodium phosphate buffer (pH 6.6; 200 mM) and 0.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 minutes and then 0.5 ml of trichloroacetate (10%) was added. After centrifugation at 650 rpm for 10 minutes, 1 ml of the supernatant was mixed to

1 ml of distilled water and 0.2 ml of ferric chloride (0.1%). The control was done by replacing formulations with distilled water. The absorbance was read at 700 nm. The ion reducing power was expressed as percentages and calculated as follows:

$$\text{Reducing } (\%) = \left[\frac{(\text{DOcontrol} - \text{DOsample})/}{\text{DOcontrol}} \right] \times 100$$

Metal chelating. The metal chelating properties were assessed by the Cu^{2+} and Fe^{2+} chelating through the lipoperoxidation and hemolysis assays respectively.

Lipoperoxidation assay. The lipoperoxidation assay was evaluated by the method of Okhawa et al.[30] A volume of 150 μl of each formulations (1.25; 2.5; 3.75; and 5 mg/ml) was added to 50 μl of an emulsion of olive oil (olive oil [10], phosphate buffer [10 mM, pH 7], and tween [20%]). A volume of 50 μl of $FeSO_4$ (40 μM in a phosphate buffer) was added to initiate lipoperoxidation. The mixture was incubated at 37°C for 16 hours in the dark. The reaction was stopped by cooling and addition 50 μl of EDTA (20 mM) and 50 μl of vitamin C (4 mM). Then, 0.5 ml of trichloroacetic acid (20%) and 0.5 ml of thiobarbituric acid (0.78%) were added and the final mixture was incubated at 95°C for 45 minutes. After cooling at 25°C and centrifugation, the absorbance was read at 532 nm. The control was done by replacing formulations with phosphate buffer. The lipoperoxidation inhibition was calculated as follows:

$$\text{Inhibition } (\%) = \left[\frac{(\text{DOcontrol} - \text{DOsample})/}{\text{DOcontrol}} \right] \times 100$$

Hemolysis assay. The hemolysis assay was evaluated by the method of Arbos et al.[31] A volume of 0.1 ml of formulations (5; 6.25; 7.5; 8.75; and 10 mg/ml) was added to 0.5 ml of NaCl (0.9%) and 50 μl of red blood cell suspension. The mixture was incubated at 37°C for 30 minutes. After incubation, 50 μl of $CuSO_4$ (0.1 M) was added and the mixture was reincubated at 37°C for 30 minutes. The absorbance of the supernatant was read at 540 nm. The control was done without the formulations. The hemolysis inhibition was calculated as follows:

$$\text{Inhibition } (\%) = \left[\frac{(\text{DOcontrol} - \text{DOsample})/}{\text{DOcontrol}} \right] \times 100$$

For the 2 metal chelating assays, the Inhibition Concentration (IC_{50}) was calculated.

Antihyperglycemiant properties

The antihyperglycemiant properties of the formulations were assessed by 3 mechanisms: glucophagic effects, cellular glucose uptake stimulating effects, and inhibition of carbohydrate digestion.

Glucophagic effects. The glucophagic effects were evaluated through the glucose adsorption assay.

Glucose adsorption assay. The glucose adsorption assay was done by the method of Ou et al.[32] A volume of 0.5 ml of formulations (5; 10; 15; and 20 mg/ml) was added to 0.5 ml of glucose solution (12.5, 25, 37.5, and 50 mM). The mixture was incubated at 37°C for 60 minutes. After centrifugation at 4000 g for 20 minutes, the supernatant was collected and the glucose content was determined by the method of Trinder.[33] The glucose bound was calculated as follows:

$$\text{Glucose bound} (\%) = \left[\frac{(\text{Glucose}_{\text{initial}} - \text{Glucose}_{\text{final}})/}{\text{Glucose}_{\text{initial}}} \right] \times 100$$

Stimulation of cellular glucose uptake. The cellular glucose uptake stimulating effects were evaluated through glucose uptake by yeast and muscle cell assays.

Glucose uptake by yeast cells. Yeast cells suspension was prepared by the method of Cirillo.[34] A volume of 0.5 ml of formulations (2.5; 5; 7.5; and 10 mg/ml) was added to 0.5 ml of glucose solution (25 mmol/l). The mixture was incubated at 37°C for 10 minutes. Then 50 μl of yeast suspension was added to start the reaction. The mixture was vortexed, and incubated at 37°C for 60 minutes. After incubation, the mixture was centrifuged at 3000 g for 5 minutes. The supernatant was collected and the glucose content was determined by the method of Trinder.[33] The control was done without the formulation. The glucose uptake was calculated as follows:

$$\text{Glucose uptake} (\%) = \left[\frac{(\text{Abs control} - \text{Abs sample})/}{\text{Abs control}} \right] \times 100$$

Glucose uptake by muscle cells. Glucose uptake of the formulation by muscle cells was evaluated by the method of Al-Awadi et al.[35] Muscle tissue obtained from psoas muscle of adult rats was cut into pieces of 0.25 g, and preincubated in a Krebs solution containing glucose (11.1 mM) for 5 minutes in the CO_2 incubator. Three mediums were done including muscle tissue alone (Control), muscle tissue with insulin (50 mU/l), and muscle tissue with both insulin and formulation (5, 7.5, and 10 mg/ml). Each medium was incubated for 2.5 hours in a CO_2 incubator. Aliquots of 2 ml were removed from each medium after 30 minutes of incubation, and glucose content was determined by the method of Trinder.[33] Glucose uptake was calculated as follows:

$$\text{Glucose uptake} (\%) = \left[\frac{(\text{Abs control} - \text{Abs sample})/}{\text{Abs control}} \right] \times 100$$

Statistical analysis

Statistical analysis was done by the Statistical Package for Social Science software, version 20.0 for Windows. One-way

Table 1. Phytochemicals content of formulations.

PHYTOCHEMICALS	F1	F2	F3
Total polyphenols (µg EGA/mg of F)	487.11 ± 4.02[a]	351.11 ± 5.75[b]	485.78 ± 4.82[a]
Flavonoids (µg EC/mg of F)	280.86 ± 0.18[a]	150.57 ± 0.11[b]	203.44 ±031[c]

Abbreviations: EC, equivalence of Catechin; EGA, equivalence of Gallic acid; F, formulation.
Values assigned with different letter sin the same line are significantly different at $P < .05$ according to LSD.

Table 2. SC_{50} of different radicals.

SC_{50} (MG/ML)	F1	F2	F3
DPPH•	2.75[a]	2.97[b]	3.51[c]
ABTS•	2.6[a]	2.67[b]	2.76[c]
NO•	3.3[a]	2.59[b]	3.29[a]

Abbreviation: F, formulation; SC, scavenging concentrations.
Values assigned with different letters in the same line are significantly different at $P < .05$ according to LSD.

Analysis of variance between groups was done with Least Significant Difference. A Post hoc test was realized to compare values 2 by 2. A significativity level was retained at .05. The SC_{50} and IC_{50} values were Oed by linear regression.

Results

Polyphenolic compounds content of formulations

The polyphenolic compounds content of formulations is shown in Table 1. Total polyphenols content was varied to 351.11 at 487.11 µg EGA/mg of Formulation, while the flavonoids content was varied to 150.57 at 280.86 µg EC/mg of Formulation. F1 contains the most total polyphenols and flavonoids, while F2 contains the least of both.

Antioxidant properties

Scavenging radicals. The DPPH•, ABTS•, and NO• scavenging properties of different formulations are shown in Table 2 through their SC_{50}. All formulations showed scavenging activities on the 3 radicals. The SC_{50} for the DPPH• radical ranges from 2.75 mg/ml (F1) to 3.51 mg/ml (F3), for the ABTS• radical from 2.6 mg/ml (F1) to 2.76 mg/ml (F3), and for the NO• radical from 2.59 mg/ml (F2) to 3.3 mg/ml (F1). F1 has the highest DPPH• and ABTS• scavenging activities and F2 has the highest NO• scavenging activity.

Reducing property. The abilities of formulations to reduce the MoO_4^{2-} and Fe^{3+} are shown in Table 3. All formulations showed a total antioxidant capacity (TAC) in a manner proportional to their concentrations. TAC varied between 1.75 and 2.72 mg ET/mg of formulation for F1, 1.58 and 2.15 mg ET/mg of formulation for F2, and 2.5 and 3.55 mg ET/mg of formulation for F3. F3 has the highest TAC, while F2 has the lowest.

The Fe^{3+} reducing capacity is expressed as a percentages of iron reducing and varied from 5.53% to 22.81%. F1 has the highest iron reducing capacity, while F3 has the lowest.

Metals chelating. The abilities of formulations to inhibit lipoperoxidation by chelating Fe^{2+} and hemolysis by chelating Cu^{2+} are shown in Table 4 through their IC_{50}. All formulations showed inhibiting activities on lipoperoxidation and hemolysis. The IC_{50} varies from 2.53 mg/ml (F1) to 3.17 mg/ml (F2) for lipoperoxidation and from 2.57 mg/ml (F2) to 4.08 mg/ml (F3) for hemolysis. F1 has the highest lipoperoxidation inhibitory activity and F2 has the highest hemolysis inhibiting activity, while F2 and F3 have respectively the lowest lipoperoxidation and hemolysis inhibiting activities.

Antihyperghycemiant properties

Glucophagic properties. The glucophagic properties of formulations were evaluated through the glucose adsorption capacity (Figure 1). The different formulations adsorbed glucose in a dose-dependent manner. The percentages of glucose adsorption varied from 77.93% to 82.43% for F1, 76.43% to 87.01% for F2, and 73.83% to 83.62% for F3. F2 showed the best glucophagic potential.

Glucose uptake by yeast cells. The effects of formulations on glucose uptake by yeast cells are shown in Figure 2. The different formulations stimulated glucose uptake by the yeast cells in a concentration-dependent manner. The percentages of glucose uptake for F1 ranged from 31.9%, 45.18%, 35.51%, and 50.71% for F2, and 36.69% to 50.45% for F3. F3 and F2 showed the best glucose uptake by the yeast cells.

Glucose uptake by muscle cells. The effects of formulations on glucose uptake by yeast cells are shown in Table 5. Insulin alone resulted in a muscle glucose uptake of 11.89%. However, the different formulations at all concentrations significantly increased muscle glucose uptake compared to insulin alone, with uptake rates ranging from 33.28% to 39.45% for F1, from 32.26% to 35.52% for F2, and from 21.81% to 23.43% for F3. F1 was the most efficient in glucose uptake by muscle cells.

Discussion

One of the ways to combat chronic non-communicable diseases and many infectious diseases would be to act on

Table 3. Reducing capacities of formulations.

ASSAYS	TOTAL ANTIOXIDANT CAPACITY MG ET/G OF FORMULATION					Fe^{3+} REDUCING (%)
(FORMULATIONS)	5 MG/ML	6.25 MG/ML	7.5 MG/ML	8.75 MG/ML	10 MG/ML	1 MG/ML
F1	1.75 ± 0.01[a]	2.21 ± 0.01[a]	2.37 ± 0.01[a]	2.66 ± 0.01[a]	2.72 ± 0.03[a]	22.81 ± 0.9[a]
F2	1.58 ± 0.01[b]	1.71 ± 0.01[b]	1.78 ± 0.01[b]	1.93 ± 0.01[b]	2.15 ± 0.01[b]	13.211 ± 2.4[b]
F3	2.5 ± 0.01[c]	3.01 ± 0.01[c]	3.24 ± 0.03[c]	3.5 ± 0.01[c]	3.55 ± 0.03[c]	6.05 ± 1.7[c]

Abbreviations: ET, equivalent trolox; F, formulation.
Values assigned with different letters in the same column are significantly different at $P < .05$ according to LSD.

Table 4. IC_{50} of lipoperoxidation and hemolysis.

IC_{50} (MG/ML)	F1	F2	F3
IC_{50} lipoperoxidation	2.53[a]	3.17[b]	2.78[c]
IC_{50} hemolysis	3.12[a]	2.57[b]	4.08[c]

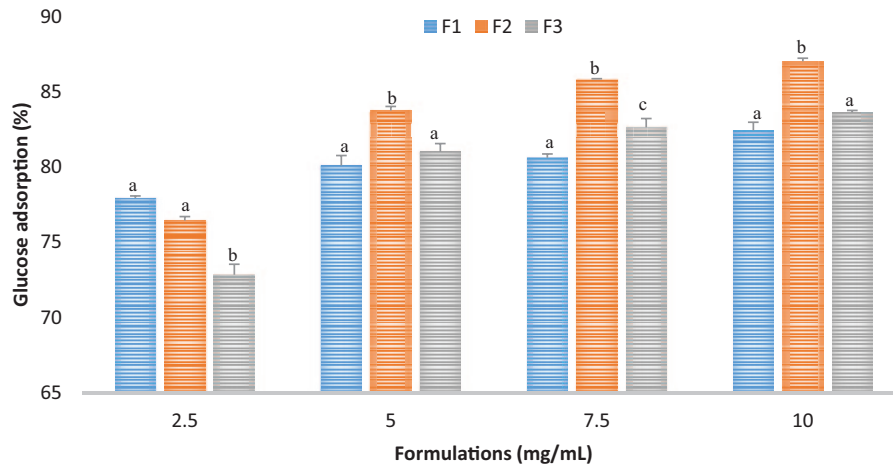Abbreviations: F: formulation; IC, inhibition concentrations.
Values assigned with different letters are significantly different at $P < .05$ according to LSD.

the metabolic disorders common to these different diseases, including oxidative stress and hyperglycemia. This study aimed to evaluate the antioxidant and antihyperglycemiant properties of EcXaPu, EcXa, and EcPu.

Since polyphenols in general and flavonoids, in particular, are strongly involved in the antihyperglycemic and antioxidant properties of plants,[36,37] we started this work by quantifying their contents in the 3 formulations. The different formulations had a considerable levels of total polyphenols and flavonoids. However, there were very large differences between the different formulations, which could be explained by their composition, the constituent plants, or even the proportions of plant extracts used. Subsequently, we evaluated the antioxidant properties of the formulations by 3 mechanisms: free radical scavenging, reducing, and metal chelating. The radicals DPPH•, ABTS•, and NO• were trapped by the formulations. These results can be due to the flavonoids content in our formulations. The redox potential of the OH groups of flavonoids gives them the ability to transfer a proton and/or an electron, thus trapping the free radicals. The antiradical strength of our formulations corroborates with the studies of Deepak and Anurekha and Anurekha,[38] who had already shown that the combination of 4 extracts of plants had antiradical activities against DPPH. Kabilan et al[39] also showed the same effects with their polyherbal formulation, while Chanthasri et al[40] showed the antiradical activities against DPPH and ABTS of 20 polyherbal formulations. Prety and Surech[41] also showed that HP-4, a formulation made from 4 medicinal plants, had antiradical activities against NO•. This ability to transfer electrons was confirmed in the second mechanism, which consisted of evaluating reducing power through the ability of the formulations to reduce the MoO_4^{2-} and Fe^{3+} ions. The various formulations resulted in a reduction of these 2 ions. These results

can still be attributed to the flavonoids content of the formulations. Khan et al[42] have already demonstrated the capacity of flavonoids to reduce MoO_4^{2-}. On the other hand, numerous formulations of medicinal plants have already proved their reducing properties, as in Gupta et al[43] and Kajaria et al[44] which exhibited an iron reducing power. All formulations also chelated Fe^{2+} and Cu^{2+}, thus inhibiting lipoperoxidation and hemolysis, respectively. These results would be due to the ability of the phenolic compounds contained in these formulations to chelate iron, inhibiting the formation of the ferryl-perferryl complex, initiating the lipoperoxidation or the blocking of the EOR, thus preventing the oxidative action of the membrane lipids.[45] Indeed, flavonoids are renowned for their ability to chelate Fe^{2+} and Cu^{2+}, thus preventing lipid peroxidation.[46] This ability to protect from lipoperoxidation could account for the protection of the erythrocyte membrane, which also consists essentially of lipids. In the same sense, the N-Miracle formulation has already shown its ability to inhibit lipid oxidation due to its content in flavonoids.[47] Shanmugasundaram et al[17] showed the ability of polyherbal formulation ADPHF6 to chelate iron ions. Chanthasri et al[40] also showed the same activity with their 20 polyherbal formulations.

In the last part of our study, we evaluated the antihyperglycemic properties of the 3 formulations. Two antihyperglycemic mechanisms were investigated: glucophagic potency and cellular glucose uptake. The different formulations showed glucophagic power, materialized by the glucose adsorption rates obtained (Figure 1). This activity is thought to be due to the formation of osidic bonds following the complexation of glucose by the hydroxyl groups of flavonoids to form glycosyl flavonoids.[48] Sapadipa et al[49] have also shown the ability of a polyherbal formulation called Mehon to adsorb glucose. All 3 formulations also stimulated peripheral glucose uptake by both yeast cells (Figure 2) and muscle cells (Table 5). This would account for the ability of the polyphenols contained in our formulations to mimic the effects of insulin or to stimulate non-insulin-dependent transporters with respect to the effects observed at the level of yeast cells, which make an insulin-independent cellular uptake. Whereas the effects observed at the muscle level would account for the ability to stimulate insulin sensitivity and GLUT 4 translocation, thus increasing muscle glucose uptake. Gaikwad et al[12] had already noted the

Figure 1. Glucose adsorption capacities of formulations.
Abbreviation: F, formulation.
Values assigned with different letters are significantly different at $P < .05$ according to LSD.
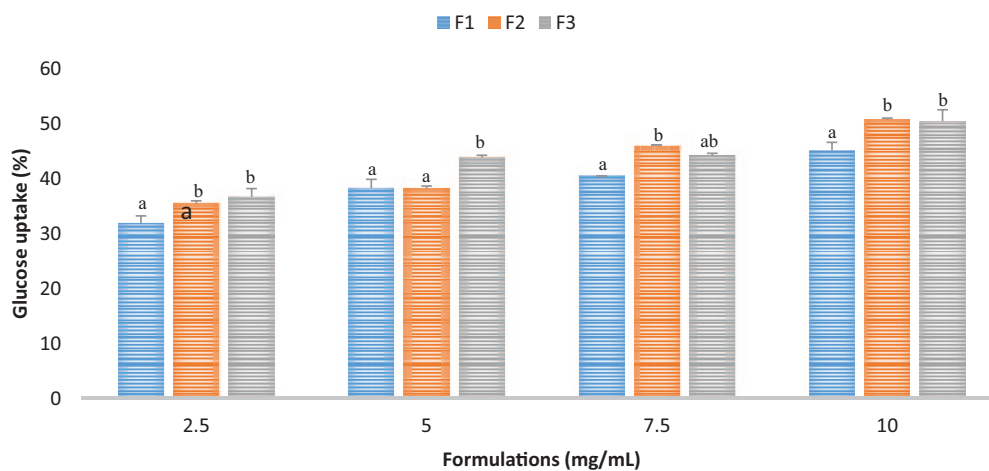
Figure 2. Glucose uptake by yeast cells.
Abbreviation: F, formulation.
Values assigned with different letters are significantly different at $P < .05$ according to LSD.

Table 5. Glucose uptake by muscle cells.

GLUCOSE UPTAKE BY MUSCLE CELLS (%)			
Muscle tissue	0*		
Muscle tissue + insulin	11.89		
[Formulations]	5 mg/ml	7.5 mg/ml	10 mg/ml
Formulation 1	33.28 ± 3.03*a	34.75 ± 2.51*a	39.45 ± 2.78*a
Formulation 2	32.26 ± 2.18*b	32.44 ± 1.75*b	35.52 ± 2.01*b
Formulation3	21.81 ±1.81*c	22.08 ±2.61*c	23.43 ±2.03*c

Values assigned with different letters in the same column are significantly different at $P < .05$ according to LSD.
*$P < .05$: significantly different in comparison to MT + insulin.

ability of polyphenols to decrease hyperglycemia by cellular glucose uptake following an increase in insulin sensitivity. Riaz et al[50] have shown the ability of a polyherbal formulation to stimulate glucose uptake by yeast cells. While Paddy et al[51] showed the stimulation of muscle glucose uptake by a polyherbal formulation.

Conclusion

The 3 polyherbal formulations (EcXaPu, EcXa, and EcPu) showed both antioxidant and antihyperglycaemic properties and acts through different mechanisms. This demonstrates the value of combining plant extracts to achieve better effects for certain diseases.

REFERENCES

1. World Health Organization (WHO). Non communicable diseases. 2021. Accessed January 18, 2022. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases
2. Le N-A. Postprandial triglycerides, oxidative stress, and inflammation, apolipoproteins, triglycerides and cholesterol. *Intech Open*. 2020.
3. Ma X, Chen Z, Wang L, et al. The pathogenesis of diabetes mellitus by oxidative stress and inflammation: its inhibition by berberine. *Front Pharmacol*. 2018;9:782.
4. Giri B, Dey S, Das T, Sarkar M, Banerjee J, Dash SK. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: an update on glucose toxicity. *Biomed Pharmacother*. 2018;107:306-328.
5. Ivanov AV, Bartosch B, Isaguliants MG. Oxidative stress in infection and consequent disease. *Oxid Med Cell Longev*. 2017;2017:1-3.
6. Forcados GE, Muhammad A, Oladipo OO, Makama S, Meseko CA. Metabolic implications of oxidative stress and inflammatory process in SARS-CoV-2 pathogenesis: therapeutic potential of natural antioxidants. *Front Cell Infect Microbiol*. 2021;11:654813.
7. Lima-Martínez MM, Carrera Boada C, Madera-Silva MD, Marín W, Contreras M. COVID-19 and diabetes: a bidirectional relationship. *Clin E Investig*. 2021;33:151-157.
8. Ntyonga-Pono M-P. COVID-19 infection and oxidative stress: an underexplored approach for prevention and treatment? *Pan Afr Med J*. 2020;35:1-2. doi:10.11604/pamj.supp.2020.35.2.22877
9. Fonkoua M, Nguimkeng SB, Sandeu KR, et al. Antioxidant and postprandial glucose-lowering potential of the hydroethanolic extract of *Nypa fruticans* seed mesocarp. *Biol Med*. 2017;9:1-6. doi:10.4172/0974-8369.1000407
10. Takuissu NGR, Ngondi JL, Oben JO. Antioxidant and glucose lowering effects of hydroethanolic extract of *Baillonella toxisperma* pulp. *J Food Res*. 2020;9:20.
11. Kim Y, Keogh JB, Clifton PM. Polyphenols and glycemic control. *Nutrients*. 2016;8:E17.
12. B. Gaikwad S, Krishna Mohan G, Rani MS. Phytochemicals for diabetes management. *Pharm Crops*. 2014;5:11-28.
13. Che CT, Wang ZJ, Chow MS, Lam CW. Herb-herb combination for therapeutic enhancement and advancement: theory, practice and future perspectives. *Molecules*. 2013;18:5125-5141.
14. Gauttam VK, Kalia AN. Development of polyherbal antidiabetic formulation encapsulated in the phospholipids vesicle system. *J Adv Pharm Technol Res*. 2013;4:108-117.
15. Aslam MS, Ahmad MS, Mamat AS, Ahmad MZ, Salam F. An update review on polyherbal formulation: a global perspective. *Syst Rev Pharm*. 2016;7:35-41.
16. Venkateswaran MR, Jayabal S, Murugesan S, Periyasamy S. Identification of polyphenolic contents, *in vitro* evaluation of antioxidant and antidiabetic potentials of a polyherbal formulation-Mehani. *Nat Prod Res*. 2021;35:2753-2757.
17. Shanmugasundaram D, Duraiswamy A, Viswanathan A, Sasikumar CS, Cherian SM, Cherian KM. Development of an antidiabetic polyherbal formulation (ADPHF6) and assessment of its antioxidant activity against ROS-induced damage in pUC19 and human lymphocytes – an *in vitro* study. *J Complement Integr Med*. 2016;13:267-274.
18. Moukette BM, Ama Moor VJ, Biapa Nya CP, et al. Antioxidant and synergistic antidiabetic activities of a three-plant preparation used in Cameroon Folk Medicine. *Int Sch Res Notices*. 2017;2017:9501675-9501677.
19. Ibrahim LB, Idowu PF, Mose SOA, Alabi MA, Ajani EO. Antidiabetic potential of stem bark extract of *Enantia chlorantha* and lack of modulation of its therapeutic efficacy in diabetic rats co-administered with lisinopril. *Acta Chim Slov*. 2021;68:127.
20. Etoundi CB, Kuaté D, Ngondi JL, Oben J. Anti-amylase, anti-lipase and antioxidant effects of aqueous extracts of some Cameroonian spices. *J Nat Prod*. 2010;3:165-171.
21. Njateng GS, Iqbal J, Zaib S, et al. Antidiabetic potential of methanol extracts from leaves of *Piper umbellatum* L. and Persea americana Mill. *Asian Pac J Trop Biomed*. 2018;8:160-165.
22. Singleton V, Rossi J. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *AJEV*. 1965;16:144-158.
23. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and in *vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium DC. BMC Complement Altern Med*. 2010;10:21.
24. Katalinić V, Milos M, Modun D, Musić I, Boban M. Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food Chem*. 2004;86:593-600.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26:1231-1237.
26. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem*. 1982;126:131-138.
27. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol*. 1995;28:25-30.
28. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999;269:337-341.
29. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Jap J Nutr*. 1986;44:307-315.
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95:351-358.
31. Arbos KA, Claro LM, Borges L, Santos CA, Weffort-Santos AM. Human erythrocytes as a system for evaluating the antioxidant capacity of vegetable extracts. *Nutr Res*. 2008;28:457-463.
32. Ou S, Kwok K, Li Y, Fu L. *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. *J Agric Food Chem*. 2001;49:1026-1029.
33. Trinder P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. *J Clin Pathol*. 1969;22:246.
34. Cirillo VP. Mechanism of glucose transport across the yeast cell membrane. *Bacteriol J*. 1962;84:485-491.
35. Al-Awadi FM, Khattar MA, Gumaa KA. On the mechanism of the hypoglycaemic effect of a plant extract. *Diabetologia*. 1985;28:432-434.
36. Hodaei M, Rahimmalek M, Arzani A, Talebi M. The effect of water stress on phytochemical accumulation, bioactive compounds and expression of key genes involved in flavonoid biosynthesis in *Chrysanthemum morifolium* L. *Ind Crops Prod*. 2018;120:295-304.
37. Aryaeian N, Sedehi SK, Arablou T. Polyphenols and their effects on diabetes management: a review. *Med J Islam Repub Iran*. 2017;31:134.
38. Deepak J, Anurekha J. Development of polyherbal with antioxidant activity. *Asian J Pharm Clin Res*. 2018;11:483-484.
39. Kabilan S, Baskar R, Poorani G. Polyherbal formulation as potent nutraceutical: antioxidant and antibacterial activity. *Int J Eng Adv Technol*. 2019;9:803-806.
40. Chanthasri W, Puangkeaw N, Kunworarath N, et al. Antioxidant capacities and total phenolic contents of 20 polyherbal remedies used as tonics by folk healers in Phatthalung and Songkhla provinces, Thailand. *BMC Complement Altern Med*. 2018;18:73.
41. Preeti P, Suresh N. *In vitro* antioxidant potential of a herbal preparation containing four selected medicinal plants. *J Krishna Inst Med Sci Univ*. 2012;1:52-63.
42. Khan RA, Khan MR, Sahreen S, Ahmed M. Assessment of flavonoids contents and *in vitro* antioxidant activity of *Launaea procumbens*. *Chem Cent J*. 2012;6:43.
43. Gupta M, Karmakar N, Sasmal S. *in vitro* antioxidant activity of aqueous and alcoholic extracts of polyherbal formulation consisting of *Ficus glomerata* roxb. And *Symplocos racemosa* Roxb. Stem bark assessed in free radical scavenging assays. *Int J Pharmacogn Phytochem Res*. 2017;9:181-189.
44. Kajaria DK, Gangwar M, Sharma AK, Tripathi YB, Tripathi JS, Tiwari S. Evaluation of *in vitro* antioxidant capacity and reducing potential of polyherbal drug-bhāraṅgyādi. *Anc Sci Life*. 2012;32:24-28.
45. Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncol*. 2012;2012:137289-137321.
46. Rahal A, Kumar A, Singh V, et al. Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomed Res Int*. 2014;2014:761264-761319.
47. Meenatchi S, Jeyaprakash K. Screening of phytochemical and *in vitro* antioxidant property of N-miracle (polyherbal formulation). *World J Pharmaceut Res*. 2015;4:1702-1717.
48. Li SS, Wu J, Chen L-G, et al. Biogenesis of C-glycosyl flavones and profiling of flavonoid glycosides in Lotus (*Nelumbo nucifera*). *PLoS One*. 2014;9:e108860.
49. Paul S, Majumdar M. in-vitro antidiabetic propensities, phytochemical analysis, and mechanism of action of commercial antidiabetic polyherbal formulation "Mehon". *Proceedings*. 2020;79:7.
50. Riaz Z, Ali MN, Qureshi Z, Mohsin M. *in vitro* investigation and evaluation of novel drug based on polyherbal extract against type 2 diabetes. *J Diabetes Res*. 2020;2020:1-9.
51. Paddy V, Tonder J, Steenkamp V. *In vitro* antidiabetic activity of a polyherbal tea and its individual ingredients. *Br J Pharm Res*. 2015;6:389-401.