



RESEARCH ARTICLE

ANTIHYPERGLYCEMIC AND ANTI-OXIDANT POTENTIAL OF ETHANOL EXTRACT OF *VITEX THYRSIFLORA* LEAVES ON DIABETIC RATS

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Abstract

Objective: *Vitex thyriflora* Baker (Verbenaceae) is a glabrous under shrub that is widely distributed in Cameroon. Diabetes is characterized by chronic hyperglycemia, a source of increased oxidative stress and tissue oxidative damage. The objective of current study was to estimate the antihyperglycemic and anti-oxidant potential of ethanol extract of *Vitex thyriflora* leaves on diabetic rats

Methods: The antihyperglycemic effect of an ethanol extract of *Vitex thyriflora* leaves was investigated in normal male rats and streptozotocin induced diabetic male rats and its antioxidant potential was evaluated. After preparation of the extract, it was subjected to a phytochemistry screening, and tested on male rats made hyperglycemic in the Oral Glucose Tolerance Test (OGTT) and in streptozotocin diabetic rats. Glibenclamide (10 mg/Kg) was served as a positive control in both experiments. The evaluation of the antioxidant potential was done through the determination of the total polyphenols and total flavonoid contents, and by using the ferric reducing antioxidant power assay and the free radical scavenging activity on DPPH method.

Results: The results show that the extract contains alkaloids, flavonoids, steroids, saponins and phenolic compounds. The test on hyperglycemic rats in OGTT showed that, the extract was effective (at a dose of 200 mg/Kg) to significantly decreased glucose-induced hyperglycemia (** $p < 0.01$). The extract was ineffective on streptozotocin induced diabetic rats. The study of the antioxidant potential showed that, polyphenols and flavonoids increase with the concentration of the extracts. IC₅₀ value was found to be 65.97, based on the log (inhibitor) vs. normalized response-Variable slope.

Conclusion: FRAP appears to be significantly highly correlated with total polyphenols content and total flavonoids content. This shows that the ethanol extract of *V. thyriflora* leaves could be served to prevent acute hyperglycemia, but not a chronic hyperglycemic state.

Keywords: Antihyperglycemic, antioxidant, diabetic rats, *Vitex thyriflora*.

INTRODUCTION

The genus *Vitex* (Verbenaceae) is constituted by 250 species of small trees and shrubs which occur in tropical and subtropical regions¹. *Vitex thyriflora* Baker (Verbenaceae) is a glabrous under shrub that is widely distributed in Cameroon². This plant is reported to be useful in the treatment of orchitis². The root barks are used in the treatment of stomach pains, sexual sterility and wounds³. Previous chemical investigations on the leaves and fruits resulted in the isolation of

phytoedysteroids³. However, the investigation of some other *Vitex* species have resulted in the isolation of iridoids^{4,5,6}, diterpenes^{7,8}, steroids⁹, flavonoids⁶ and triterpenoids⁹. Several of previous compounds isolated from *Vitex* genus have shown antioxidants activities^{10,11}. Among all secondary metabolites, phenolic antioxidants appear to be the most important since they have shown promising antioxidant activity in both *in vivo* and *in vitro* investigations. Plant phenolics are mainly classified into five major groups, phenolic acids, flavonoids, lignans, stilbenes and

tannins^{12,13,14}. The antioxidant activities of these compounds have been attributed to various mechanisms which have been established by various assay procedures¹⁵. Antioxidant activity has a fundamental role in cellular protection during an inflammation process. The identification of phytochemical compounds in plant species has been exploited in recent years, due to the growing popularity of herbal medicines and consumers growing enthusiasm for foods with bioactive characteristics that aid in preventing and fighting disease^{16,17}. Diabetes is characterized by chronic hyperglycemia, a source of increased oxidative stress and tissue oxidative damage. In particular, hyperglycemia promotes the glycation of proteins resulting in the formation of advanced glycation products (AGE). Thus antioxidant molecules appear as an opportunity for a strategy to fight not only against hyperglycemia, but also against complications related to diabetes mellitus^{18,19}. Then, the objectives of this study based on these findings, was first to evaluate the antihyperglycemic effect of an ethanol extract of *V. thyriflora* leaves in glucose loaded rats and in STZ-diabetic rats and second to determine the Total Polyphenols Content (TPC), Total Flavonoids Content (TFC), the Ferric Reducing Antioxidant Power Assay (FRAP) and the DPPH free radical scavenging assay of this extract.

MATERIALS AND METHODS

Experimental

Drugs and Chemicals

Streptozotocin, quercetin and catechin were obtained from Sigma Chemicals (St. Louis, MO). Glibenclamide (Glycomin®) was obtained from Strides Aroclat Ltd. Bangalore, India. All other chemicals used were of analytical grade. Spectrophotometric measurements were done using the equipment available at Institute of Medical Research and Medicinal Plant Studies.

Plant material

The leaves of *V. thyriflora* Baker (Verbenaceae) were collected at Melong, in the Littoral Region of Cameroon in November 2009, authentication was performed by Mr. Nana who compared it with a Voucher Specimen (No 34861 HNC), in the Cameroon National Herbarium, Yaoundé.

Extraction

The extract was obtained by maceration of air-dried and powdered leaves of *V. thyriflora* (3.0 Kg) with EtOH (3x15 L, 72 h) at room temperature, to obtain a crude extract (150 g).

Phytochemical screening of secondary metabolites

The *V. thyriflora* leaves ethanol extract was also subjected to phytochemical analysis according to the methods of Harborne²⁰ and Evans²¹.

Experimental Animal

Healthy adult male wistar rats weighing 200-250 g were used in the present study. The animals were housed in clean grill cages and maintained in a well ventilated temperature controlled room at the animal house of Institute of Medical Research and Medicinal Plants studies, Yaoundé, Cameroon, with a constant 12h light/dark schedule. The animals were fed with

standard rat pellet diet and clean drinking water was made available *ad libitum*.

Induction of diabetes

Rats were fasted overnight (16h) before inducing diabetes with streptozotocin. Streptozotocin was prepared in freshly prepared sodium chloride solution 0.9% and was injected intraperitoneally at a concentration of 55 mg/Kg body weight in a volume of saline of about 500 µl/200 g body weight by applying the protocol of Szkudelski²². Control rats were injected with saline solution only. The diabetic state was confirmed 72h after streptozotocin injection. Threshold value of fasting blood glucose was taken as ≥ 200 mg/dl. Diabetic rats were weighed, matched for body weight and divided into 5 groups consisting of 5 animals each.

Antihyperglycaemic effect of ethanol extract of *V. thyriflora* leaves on diabetic rats

The approval of the Institutional Animal Ethics Committee was obtained before starting the study. An International protocol for conducting experiments on animals were followed.

Experimental design

The Diabetic rats were divided into 5 groups with five rats each: (A, B, C and D). Group A rats received DMSO 3%, those of group B and C were treated with ethanol extract of *V. thyriflora* at the doses of 200 and 300 mg/Kg body weight respectively, and Group D received glibenclamide (10 mg/Kg body weight). Blood samples were collected before the commencement of treatment with the extract and then after, at 1h, 3h and 5h intervals.

Oral Glucose Tolerance Test on normoglycemic rats

This study was carried out on normal male rats with normal blood glucose level, according to the method of Schoenfelder *et al.*,²³. The animals were fasted for 16 h prior to the study. Five groups with 5 animals each were constituted and the animals received a dose of 3 g/Kg of glucose by oral route 60 min after haven been treated with the extract as follows:

Group I: vehicle (DMSO 3%; 10 ml/Kg b. w, negative control),

Group II: water-ethanol extract (200 mg/Kg b.w),

Group III: ethanol extract (300 mg/Kg b. w),

Group IV: ethanol extract (400 mg/Kg b. w)

Group V: glibenclamide (10 mg/Kg b. w), was served as positive control.

Blood was collected from the animals before administration of the extract (-60 min), and then after at 0, 30, 60, 90 and 150 min post administration.

Blood Glucose Estimation

Blood samples were obtained by tail prick and fasting blood glucose levels were estimated with a One Touch Ultra glucometer (Life Scan, Inc., Milpitas, CA, USA) in all animals. Blood glucose levels were expressed in mg/dL.

Dosage of phenolic compounds and antioxidants evaluation

Determination of Total phenolic content (TPC)

The ability of the extracts to reduce the phosphomolybdic-tungstate chromogene in *Folin Ciocalteu* with maximum absorbance at 760 nm (Total phenolic content, TPC) was assessed as earlier

described by Vinson *et al.*,²⁴. Data were reported as mean±SD for triplicate measurements. Catechin was used as control and the results were expressed as mg Catechin equivalent/g (mg CE/g).

Determination of Total flavonoid content (TFC)

The Total Flavonoid Content (TFC) was measured as earlier described²⁵. Total Flavonoid Content of the extract were expressed as mg Quercetin Equivalent/g (mg QE/g) through the calibration curve with quercetin. Data were reported as mean±SD for triplicate measurements.

Determination of ferric reducing antioxidant power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) of an extract measures the ability of the extract to reduce ferric tripyridyltriazine to ferrous tripyridyltriazine, yielding a blue coloration with maximum absorbance at 593 nm. FRAP assay was performed like previously described by Benzie and Strain²⁶. The FRAP value was calculated and expressed as mg Catechin Equivalent/g (mg CE/g) based on a plotted calibration curve, using Catechin as standard, at a concentration ranging from 50 to 600 µmol.

DPPH free radical scavenging assay

The ability of the extract to scavenge free radicals by converting DPPH (purple in color) into di-phenyl hydrazine (yellow in color) was measured at 517 nm as earlier described²⁷.

The percentage (%) radical scavenging effect (RSE) of extract was calculated as follows:

$$\%RSE = \frac{Abs1 - Abs2}{Abs1} \times 100$$

Where Abs1 is the absorbance of the control (containing all reagents except for the extract), and Abs2 is the absorbance of plant extract (is the optical density in the presence of the extract). All tests were performed in triplicates. The parameter IC₅₀ (concentration at which the DPPH radicals were

scavenged by 50%) (mg/mL) was also calculated. IC₅₀ values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. The data was presented as mean values± standard deviation (n=3).

Statistical Analysis

Results were expressed as mean± SD. Statistical analysis were carried out using one way Analysis of variance (ANOVA) followed by Dunnet test for comparison to vehicle control or followed by Newman-Keuls Multiple Comparison Test for antioxidant parameters, using GraphPad Prism 5.03 software. A value of $p \leq 0.005$. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ was considered to be significant.

RESULTS

Phytochemical screening revealed that the ethanol extract of *V. thyrsoiflora* leaves contained alkaloids, flavonoids, steroids, phenolics compound, saponosids, catechic tanins and anthraquinones (Table 1). The Area Under Curve (AUC) associated with the effect of ethanol extract of *V. thyrsoiflora* leaves on Oral Glucose Tolerance Test (OGTT) in normoglycemic male rats was significantly decreased (** $p \leq 0.01$) at 200 mg/Kg, when compared to vehicle control group (474±26.93 to 388.60±16.05), as well as in the group receiving glibenclamide at 10 mg/Kg, where the AUC was decreased from 474±26.93 to 392.50±19.45 (Figure 1b). The administration of ethanol extract of *V. thyrsoiflora* leaves at 200mg/Kg or 300mg/Kg to diabetic rats didn't show any difference in the blood glucose level, according to the AUC associated with each group tested. Even glibenclamide didn't show a significant effect on lowering the high blood glucose level state induces by streptozotocin (Figure 2).

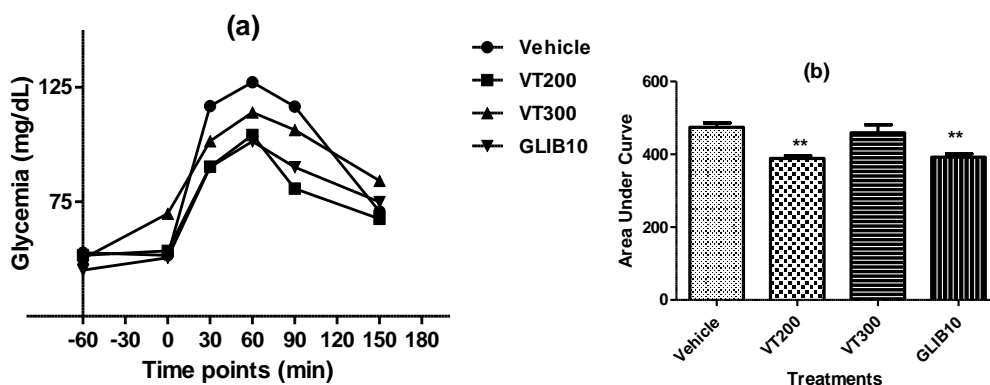


Figure 1: a. Effect of ethanol extract of *V. thyrsoiflora* leaves on glycaemia during Oral Glucose Tolerance Test (OGTT) and b. Area under curve associated with this effect of ethanol extract of *V. thyrsoiflora* leaves.

Data are expressed as means ± S.D (n=5). ** $p \leq 0.01$ compared with the corresponding value for vehicle control rats. VT200: *V. thyrsoiflora* (200 mg/Kg); VT300: *V. thyrsoiflora* (300 mg/Kg); Glib10: Glibenclamide (10 mg/Kg).

The Total Polyphenol Content (TPC) appears to be significantly dose dependent on the concentrations of the extract, until 40 mg/Kg (79.36±0.3717 mg Catechin/g), where the TPC became stable and not different to that of 50 mg/Kg (Figure 3). The Total Flavonoid Content (TFC) evaluated in *V. thyrsoiflora* ethanol extract showed a significant dose dependent increase when compared to 2.5 mg/Kg. The content

was the same at doses of 5 mg/Kg and 10 mg/Kg (Figure 4).

The correlation between Ferric Reducing Antioxidant Power (FRAP) and Total Flavonoid Content (TFC) showed a very strong positive correlation (Pearson $r=0.9223$ with $R^2=0.8507$, $p^{**} = 0.0088$) (Figure 5). The correlation between FRAP and TPC was significantly high ($p^{**}=0.0010$) (Figure 6).

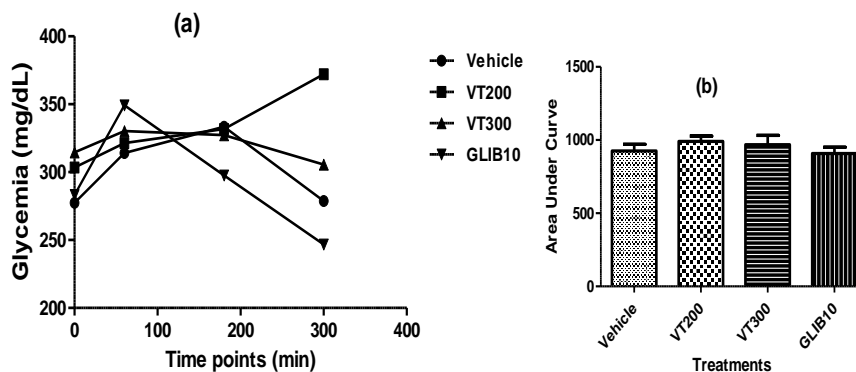


Figure 2: a. Effect of *V. thyrselflora* ethanol extract on glycemia of STZ-induced diabetic rats; b. Area under curve associated with the effect of ethanol extract of *V. thyrselflora* leaves on glycemia of STZ-induced diabetic.

Data are expressed as means ± S.D (n = 5). $p \leq 0.001$ compared with the corresponding value for vehicle control rats. VT200: *V. thyrselflora* (200 mg/Kg); VT300: *V. thyrselflora* 300 mg/Kg); Glib10: Glibenclamide (10 mg/Kg).

Table 1: Phytochemical screening of ethanol extract of *V. thyrselflora* leaves.

Phytochemical constituents tested	Results
Alkaloids	+
Flavonoids	+++
Tri Terpenoids	-
Steroids	+++
Phenolics compound	+++
Saponosids	++
Catechic tanins	+++
Anthraquinones	++
Glucosides	-

(+) = indicates presence of phytochemicals and (-) = indicates absence of phytochemicals. +++ = shows high concentration; ++ = shows moderate concentration; + = shows small concentration.

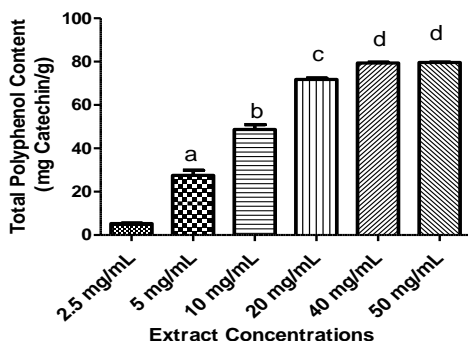


Figure 3: Total Polyphenol Content of ethanol extract of *V. thyrselflora*.

Data are expressed as means ± S.D (n=3); a significantly different from 2.5 mg/ml, b significantly different from 2.5 mg/ml and 5 mg/ml, c significantly different from 2.5 mg/ml, 5 mg/ml and 10 mg/ml, d significantly different from 2.5 mg/ml, 5 mg/ml, 10 mg/ml and 20 mg/ml.

DISCUSSION

The aim of this work was to evaluate the antioxidant and the antihyperglycemic potential of the ethanol extract of *V. thyrselflora* leaves. The phytochemical screening reveals that the ethanol extract of *V. thyrselflora* leaves possesses alkaloids, flavonoids, steroids, phenolic compounds, catechic stanins, anthraquinones and saponosids in traces. The evaluation of the effect of *V. thyrselflora* ethanol leaves extract on hyperglycemic normal rats in OGTT showed that, the 200 mg/Kg dose was the most active to lower

the AUC induced by glucose loaded in normal rats male (** $p \leq 0.01$). Glibenclamide (10 mg/Kg), a widely used antidiabetic drug was effective to decrease the AUC observed in the vehicle group (** $p \leq 0.01$).

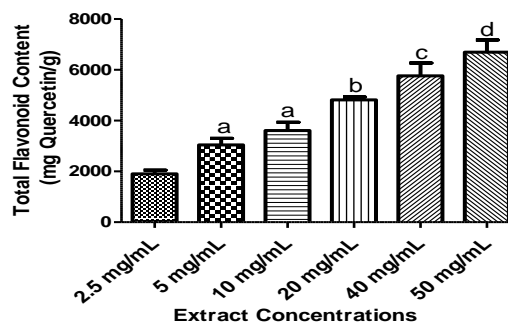


Figure 4: Total Flavonoid Content (TFC) of ethanol extract of *V. thyrselflora*.

Data are expressed as means ± S.D (n = 3); a significantly different from 2.5 mg/ml, b significantly different from 2.5 mg/ml, 5 mg/ml and 10 mg/ml, c significantly different from 2.5 mg/ml, 5 mg/ml and 20 mg/ml, d significantly different from 2.5 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml.

The results of OGTT in normal rats could be correlated with the ability of the extract to probably enhance the secretion of insulin in the likely manner of sulfonylureas and inhibit α - glucosidases present in the border brush of the small intestine^{28,29}. Enhanced tissue uptake of blood glucose induced by *V. thyrselflora* might also be taken into consideration as an alternative possibility. In current study, the decrease in blood glucose at 200 mg/Kg and not at 300 mg/Kg could be explained by the presence of hyperglycemic compounds which have become the majority at this dose. Some studies showed that, antagonistic relationship among phytochemicals would affect the efficacy of crude extracts as used in traditional medicine, like observed by Milugo *et al.*,³⁰ on antagonistic effect of alkaloids and saponin. Furthermore, the results show that administration of the extract is associated with no effect on blood glucose level in STZ-diabetic rats. Since streptozotocin selectively destroys β -cells of the pancreas, we would expect the extract to exert no effect on plasma glucose concentrations in STZ diabetic rats if the mode of

action is mediated through insulin production. Furthermore, the observations in the present study indicate that the hypoglycaemic effect of the extract as well as that of glibenclamide were far more pronounced in OGTT rats than in STZ-diabetic rats.

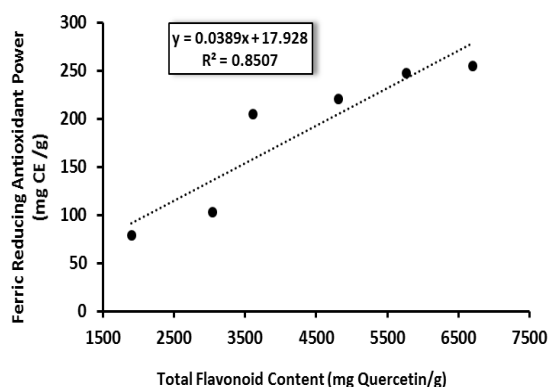


Figure 5: Correlation between FRAP and the total flavonoid content (mg QE/g).
(Pearson $r=0.9223$; $R^2=0.8507$, $p^{**}=0.0088$ ($p<0.01$)).

This result is probably in line with the early suggestion that glibenclamide was effective in moderately streptozotocin-induced diabetic animal and ineffective in severe diabetic rats^{31,32}, the extract could act in the same way as glibenclamide. These observations taken together suggest that ethanol extract of *V. thyriflora* leaves has a potential in the management of acute hyperglycaemia. Antioxidant parameters showed a dose-dependent rise in the concentration of the extract. The TPC and the TFC showed a positive correlation with the FRAP test, which could suggest that this method would act through polyphenols and flavonoids.

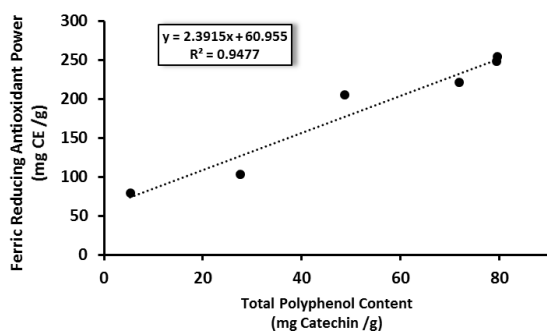


Figure 6: Correlation between FRAP and the total polyphenol content (mg QE/g).
(Pearson $r=0.9735$; $R^2=0.9477$, $p^{**}=0.0010$ ($p<0.01$)).

The Pearson correlation coefficient (R^2) is often used for measuring and describing the degree of linear regression between two continuous quantitative variables that are normally distributed. In current study, the R^2 value of the correlation between the iron reduction technique "FRAP" (Ferric Reducing Antioxidant Power) and the Total Phenolic Content was equal to 0.9477. These results suggested that a great part of the antioxidant capacity of the ethanol extract of *V. thyriflora* leaves is attributed to the Total Polyphenol Content, mainly to flavonoid content in the extract, which have the hydrogen-donor ability to reduce iron. Similar studies suggested a linear

relationship between antioxidant capacity and flavonoid contents of the plant extract^{33,34}. The DPPH assay is often used to evaluate the ability of antioxidant to donate hydrogen or to scavenge free radicals. However, DPPH scavenging activity is best represented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution³⁵. In current study IC_{50} was 65.97 mg/ml, $R^2=0.9945$ for the *V. thyriflora* leaves ethanol extract based on the log (inhibitor) vs normalized response - variable slope, which can be considered to be low antioxidant activity (IC_{50} from 50.62 to 110.46 mg/ml) according to the scale of Surinut *et al.*,³⁶; this might suggest that the antioxidant activity of this extract would not be mediated by donating hydrogen to scavenge free radicals but by the reduction of iron.

CONCLUSIONS

Based on the results of the present study, we conclude that the plant extract possesses antioxidant potential. The findings of the present study also suggest that *V. thyriflora* ethanol leaf extract could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. However, this work is the first report which evaluates antihyperglycemic effect of ethanol extract of *V. thyriflora* leaves and its antioxidant potential. Further studies should be carried out to evaluate α -amylase inhibitory and beta-glucosidase inhibitory activities isolate and identify active compounds, to understand the mechanism of action against hyperglycemia.

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AUTHOR'S CONTRIBUTION

Kowa TK: writing original draft, methodology. **Nyemb N:** formal analysis, data curation. **Tchamgoue AD:** review and editing. **Tabi TGN:** methodology, formal analysis. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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