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Chemical Profile, Cytotoxicity Anti-Radical and Hypolipidemic Activities of Tridax Procumbens of Benin.

Omédine Koukoui[1], Cokou P Agbangnan D[2]*, Esperance Medoatinsa[2], Sylvie Prigent[3], Oliver Nusse[3], Laurent Combettes[3], Dominique Sohounhloue[2].

[1]Laboratoire dePhysiologie Animale deSignalisation Cellulaireet de Pharmacologie FAST Dassa, University Polytechnique d'Abomey BP 34 Dassa Benin.
[2]Laboratoire dEtude et de Recherchen Chimie Appliquée, Unity de Recherche sur les Interactions Moléculaires (URIM/LERCA/UAC), 01BP 2009 Cotonou, Bénin.
[3]Laboratoire de Signalisation Calcique et Interactions Cellulaires dans le Foie UMRS 757 University Paris Sud France.

ABSTRACT

Tridax procumbens, a traditional medicinal plant, was analyzed in the present work for its antioxidant power through its free radicals scavenging capacity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test and its potential anti ROS (Reactiv Oxygen Species).The phytochemical screening revealed in the leaves of *Tridax procumbens* the presence of various metabolites such as flavonoids, tannins, phenols and mucilage and the absence of alkaloids, reducing sugars, leucoanthocyanins, anthocyans, anthraquinons and saponins. The antioxidant potential determination shows that *T. procumbens* has a very significant antioxidant power: IC_{50} = 0.17 mg/mL for DPPH test and C = 1mg / ml inhibit completely the production of ROS. Its high content of total polyphenols (11.49 mg/g EAG) and flavonoids (24.01 gm/g CE) could explain this activity. The toxicity test shows that the semi-ethanolic extract of *T. procumbens* is not toxic at any dose up of 10 mg / ml. This extract can be used at reasonable doses to fight against hyperlipidemia and non-communicable diseases such as hypertension, arteriosclerosis and neurodegenerative diseases.

Keywords: Phytochemical, *Tridax procumbens*, cytotoxicity, hypolipidemic, antioxidant.

Corresponding author

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INTRODUCTION

For its vital and ecological functioning, the plant synthesizes primary and secondary metabolites. Secondary metabolites in plants (fruits, vegetables, grains) involved in the protection against herbivores, microbes, or competing plants, adaptation and pollination [1]. Among the secondary metabolites we can cite the alkaloids, terpenes, phenolics. The polyphenol compounds play a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and cardioprotective and vasodilatory effects [2]. These functions have been attributed to their antioxidant activity by several mechanisms such as free radical scavengers, reducing agents, complexers of pro-oxidant metals, quenchers of the formation of singlet oxygen and stimulating the antioxidative defence enzymes activities [3].

According to the World Health Organization (WHO), about 65 to 80% of the world populations in developing countries rely on medicinal plants for their primary health care). Among these medicinal plants used by the poor populations we have *Tridax procumbens*.

Tridax procumbens Linn. (Asteraceae) is native of tropical America and naturalized in tropical Africa, Asia, and Australia. It is also found along roadsides, waste grounds, dikes, riverbanks, meadows and dunes. *Tridax procumbens* L. is a small perennial herb having short and hairy blade like leaves. Corolla is yellow in colour. Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, diarrhea, high blood pressure and to check haemorrhage from cuts, bruises and wounds and to prevent falling of hair [4]. Earlier workers have reported that it possesses antidiabetic [5], anti-bacterial [6], antiplasmodial [7], antihepatotoxic, anti-oxidant [8], antimicrobial [9], immunomodulatory [10] and anti-cancerous[11] properties. Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties [12,13]. The present study aims to assess the chemical profile, the cytotoxicity and the anti-radical potential of *Tridax procumbens* from Benin.

MATERIALS AND METHODS

Plant material

The aerial part of *Tridax procumbens* was harvested in the month of Mai, 2014 from sakete in southern of Benin. A specimen was deposited in the National Herbarium of the Department of Botany, Abomey-Calavi University. Samples were dried in shade at room temperature (25°C) until stabilization of their mass and then pulverized into coarse powder.

Biological material

Hepatocellular carcinoma cells line (HepG2 cells) were used for cytotoxicity and hypolipidemic tests whereas Human neutrophils PLB 985 cells were used for anti-radical test.

HepG2 (ATCC) were cultivated in 75 cm^2 polystyrene flasks with DMEM culture medium to which were added 10% FBS and 1% penicillin streptomycin at 37° C under athmosphere of 5% CO_2 .PLB 985 cells are human leukemic cells that are able of differentiating into neutrophils. They were grown in RPMI 1640 medium containing L-glutamine, penicillin, streptomycin and 10% FBS at 37 °C under athmosphere of 5% CO_2. They were differentiated in the presence of DMSO.

Reagents

Gallic acid, Butyl Hydroxy Anisole (BHA), quercetin and catechin were purchased from Sigma Chemical Co. (St. Louis, MO) while 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from Acros Organics (Morris Plains, NJ). All solvents used are analytical grad. DMEM medium, RPMI 1640, oleic acids, oil red O, Phorbol Myristate Acetate, luminol were purchased from Sigma, MTS-PMS reagent was obtained from Promega.

Sample Extraction

All samples were ground through a commercial coffee grinder for extraction. The mixture ethanol-water 50% (v/v) was used as extraction solvent. The extract was concentrated *in vacuo* using a rota vapor and the yield (Y) was calculated by the formula below:

$$Y\ (\%) = (\text{Mass of extract})/(\text{Mass of plant material used}) \times 100$$

Phytochemical screening

We explored the chemical potential of the leaves of *Tridax procumbens* by a series of coloring. This phytochemical screening was based on the coloring reactions and/or the precipitation reactions of the chemical compounds in plants according to the method available in literature and usually used in our laboratory [14].

Determination of phenolic compounds

Total polyphenols

The method of determination of total polyphenols according to Folin- Ciocalteu [15,16]′ consisted to use a mixture of phosphotungstic and phosphomolybdic acid which was reduced during the oxidation of phenols in the mixture of tungsten blue oxide and molybden. The absorbance was measured by a spectrophotometer (JENWAY 50/60 Hz) at 765 nm. Gallic acid was used as reference and the total polyphenol content in the extract was expressed in mg of gallic acid equivalents per gram of dry matter.

Total flavonoids

The method of aluminum trichloride ($AlCl_3$) was used to quantify the total flavonoids. This technique was based on the formation of the aluminum-flavonoids complex that had a maximum absorption at 500 nm [17,18]′.

Condensed tannins

condensed tannins dosing was achieved by the method with vanillin sulfuric [19]. The principle of this assay was based on the binding of vanillin aldehyde group on the carbon in position 6 of the ring of the catechol to form a red colored complex chromophore which absorbs at 510 nm.

Antioxidant activity

DPPH test

This activity of each extract was assessed by measuring free-radical scavenging activity via the decoloration of a solution of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). The absorbance (A) of DPPH was determined with a spectrophotometer at 517 nm [20,21]′ and the radical scavenging activity of each sample was expressed as percentage inhibition:

$$\%\ \text{inhibition} = ([A_{control} - A_{sample}]/A_{control}) \times 100$$

Where A: Absorbance

Anti-ROS test

Phorbol myristate acetate (PMA) induces the activity of NADPH oxidase and the production of free radicals in human neutrophils [22]. The production of free radicals [23] was quantified by measuring the luminescence induced by luminol with Walac 1420 plate reader. PLB 985 cells were re-suspended in ES buffer

containing 10 ug / ml of luminol, 4 U / ml of HRP and leaves extracts (1-20 µg /µl) to a final volume of 200 µl. Cells were stimulated with 100 nM PMA and ROS production was measured for 15 to 60 minutes (ROS).

Antihyperlipidemic activity

In order to induce hyperlipidemia, the method reported by Cui et al. was followed [24]. HepG2 cells (10^5) were plated on slides in normal culture medium for 24 h and the culture medium was replaced with normal culture medium (negative control cells), culture medium without FBS containing 1 mM of oleic acid (positive control) and culture medium without FBS containing 1 mM of oleic acid and *Tridax procumbens* leaves extracts at concentration of 20 mg/ml. The cells were again incubated for 24 h. After fixing the cells were treated with the ORO for 10 min before observed under microscope. The lipid droplets were colored in red and indicate lipid accumulation induced by oleic acid in HepG2 cells

Cytotoxicity

Cytotoxicity was performed by the MTS assay, according to the protocol described by Said et al [25]. HepG2 cells were plated in 96-multiwell culture plates at 1.10^5 cells per well. After 24 hours the culture medium is replaced with new medium containing extracts at various concentrations from 1mg/ml to 50mg/ml.

The cells were again incubated for 24 h. After, the reagent (MTS-PMS) was added to each well. The plate is incubated for 2 h in the dark. The absorbance at 490nm was read by a plate reader. The experiment was repeated 3 times. The percentage of viability is given by the formula:

Percentage viability = (Absorbance of treated cells / absorbance of untreated cells) x100

RESULTS AND DISCUSSION

Phytochemical Screening

Table 1: Secondary metabolites identified in *T. Procumbens*

Secondary metabolites		***T Procumbens***
Tannin	Gallic	-
	Catechic	+
Flavonoids		+
Mucilage		+
Anthocyanin		-
Leucoanthocyanin		-
Reducing sugar		+
Anthraquinones		-
Alkaloids		-
Saponosids		-
Quinone derivatives		-

As we can see in Table 1, various secondary metabolites have been highlighted in the leaves of *Tridax procumbens*. The phytochemical screening revealed the presence of catechic tannin, flavonoid, mucilage and reducing sugar whereas saponosids, Anthocyanin, Alkaloids, Anthraquinones and Quinone derivatives were absent. Our results are consistent with that of Singh [26] et al. who identified the presence of tannins and flavonoids in *Tridax procumbens*. Rajaram et al. [27] and Nirmela et al. [28] identified in the leaves of this plant harvested in Gadhinglaj Tahsil (India) the alkaloids, saponin and anthocyanin which were absent in the sample from Benin. Mukeshet and Smita [29] Reported the absence of flavonoids in the species collected from the wet lands in India. Variability of plant secondary metabolites from one region to another may depend on several factors. Among these, are the climatic and soil conditions (temperature, sun exposure, drought and salinity), storage conditions and the maturity of the plant.

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Extraction yield and phenolic content

The extraction yield of the secondary metabolites from *Tridax procumbens* leaves by the mixture ethanol-water is 9.92%.

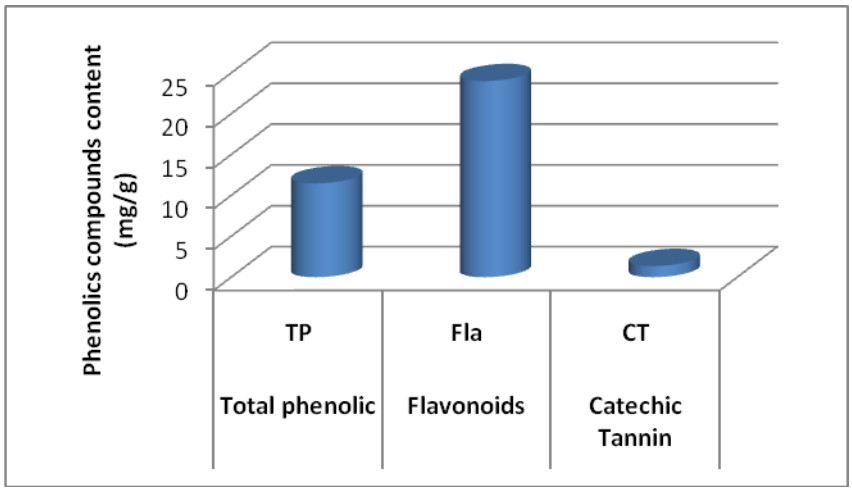

Figure 1: Phenolic content of ethanol aqueous extracts of *Tridax procumbens*

The results of the determination of total phenol (expressed as gallic acid equivalent, GAE); flavonoids (expressed in mg of quercetin equivalent per gram of dry matter) and tannin (expressed as mg catechin equivalent per gram of dry matter) shows that T. procumbens extracts have a high phenolic and flavonoids content but a low tannin content: respectively 11.49 mg/g GAE; 24.01 mg/g QE and 1.37 mg/g CE (Figure 1).

The leaves of *T. procumbens* are richer than the dates (5.66 mg/g) [30] and less rich than grape seed (40.400 mg/g) [31] considered rich in phenolic compounds. According to the work of Habila *et al*. the sample collected in Zaria (Nigeria) contain 12 mg/g GAE of total phenolic; the same value found in Benin.

Antioxidant Activity Using the DPPH· Method:

Table 2: IC_{50} of *Tridax procumbens* extract compared with those of standard

	T. procumbens	Gallic acid	BHA	Quercetin
IC_{50} (mg/mL)	0.17	0.03	0.09	0.1

Tridax procumbens has a good anti-radical activity with an IC_{50} (0.17 mg/mL) similar to that of quercetin (0.1 mg/mL). Several scientific studies conducted worldwide have shown biological activities of T. procumbens but its antioxidant power has been little evaluated. In their study of the species harvested in zaria, kaduna stade (Nigeria), Habila et al. [32] concluded that *Tridax procumbens* is a rich source of natural antioxidants.

Antioxidant Activity Using Anti-ROS test

In the positive control (col4: absence of Tp extract), PMA induces a strong production of reactive oxygen species (ROS) in phagocytes PLB985 and the maximum production was observed within 20 min. After this time, the production begins to drop up to 80 min. In the presence of PMA and different concentrations ranging from 1mg/ml to 20mg/ml of *Tridax procumbens* extracts, there is no production of ROS (Figure 2).

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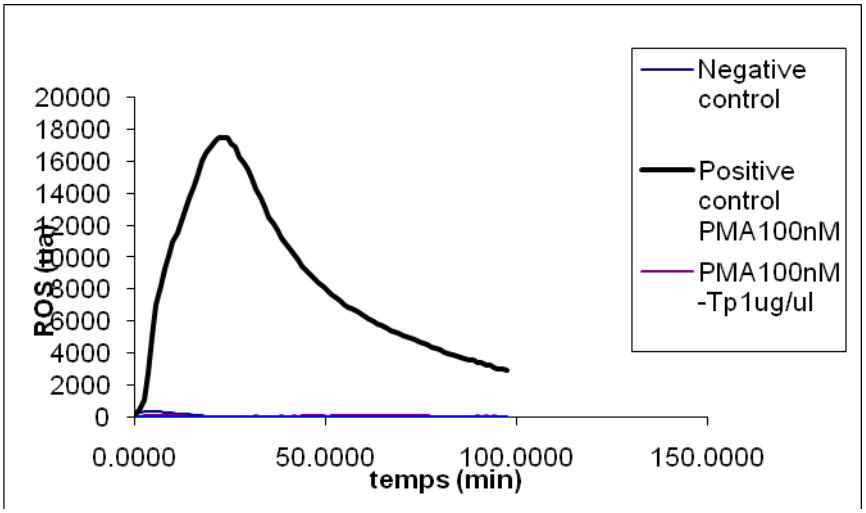

Figure 2: ROS production by phagocytes PLB985 stimulated by PMA with or without Tp extracts (1-20µm/µL)

In view of the results obtained at high concentrations, the experiment was repeated with lower concentrations of leaf extracts of Tridax procumbens (25µg/ml to 1mg/ml). ROS production induced by 100 nM PMA decreased considerably dose-depending and was canceled at 1mg/ml of extracts (Fig 3). These results are consistent with those of DPPH test and show that the leaves extract of Tp has a significant antioxidant power.

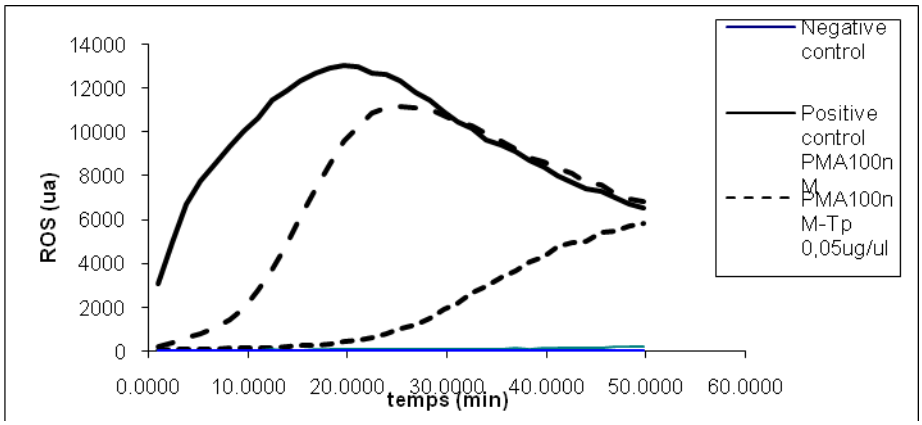

Figure 3: ROS production by phagocytes PLB985 stimulated by PMA with or without Tp extract (0,05-1µm/µL)

Antihyperlipidemic activity of *Tridax procumbens* leaves extracts:

The hypolipidemic activity of *L. taraxacifolia* leaves extracts was investigated by measuring their effect on lipid accumulation induced by oleic acid in HepG2 cells. 1um of oleic acid induced lipid accumulation (steatosis) in HepG2 cells [33]. The lipid accumulation was quantified by the "Oil Red O" which is a liposoluble lysochrome which stains in contact with lipids [34]. The presence of antioxidant molecules (leave extract of T. Procumbens) decreased significantly lipid accumulation induced by oleic acid in HepG2 cells. When the cells are treated with 20 mg/ml of extracts of T. procumbens and 1mM of oleic acid, no lipid accumulation observed in HepG2 cells.

As shown in Figures 4 and 5, the leaves extract of T. procumbens had significant effect to decrease lipid content in HepG2 cells (Figure5), compared with the model control (Figure4). In effect, hepatic lipid accumulation and oxidative stress contribute to non-alcoholic fatty liver disease (NAFLD). Thus, we hypothesized that the antihyperlipidemic and antioxidant activity of T. Procumbens leaves would attenuate events leading to NAFLD. The leaves extracts of *Tridax procumbens* may be used as prophylactic agents to prevent the induced disorders such as atherosclerosis and other.

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In conclusion, our results suggest the potential therapeutic uses of hydroethanolic extract of *Tridax procumbens* in the prevention and treatment of hyperlipidemia and related diseases.

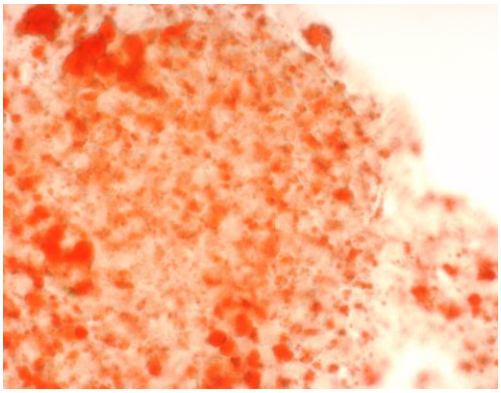

Figure 4: HepG2 cells incubated with 1mM OA without extract

Figure 5: HepG2 cells incubated with 1mM OA and 20mg/mL of T. procumbens extract.

Cytotoxicity

The MTS cytotoxicity test on HepG2 cells was used to determine the cytotoxic activities of hydroethanolic extract of *T. procumbens* leaves. In effect, many studies reported that the MTS in vitro cytotoxicity assay is a convenient method for assessing cell viability. Its ease of use, accuracy and rapid indication of toxicity are the main features found with this assay. It's a useful tool in human health risk assessment if it can be shown that this assay also has an acceptable sensitivity and specificity [35].

The concentrations of 0-10 µg/µl of hydroethanolic extract of Tridax procumbens have no effect on cell viability, but from 20 µg/µl a slight decrease in cell viability was observed (Figure5). Our results showed that the extract of leaves of T. procumbens are not cytotoxic at the concentration of 10 mg/ml and can be safely used until this therapeutic dose in the treatment of diseases.

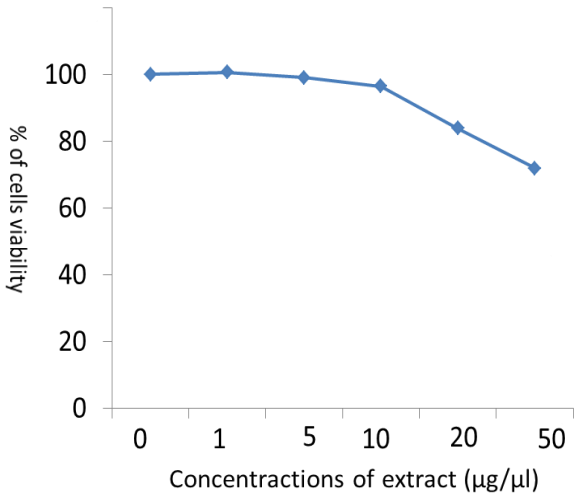

Figure 6: Dose dependent action of Tp leaves extracts on HepG2 cells viability

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper

CONCLUSION

In the present study, the hydroethanolic extract of T. Procumbens was assessed for its antioxidant potential and its hepatic lipid-lowering potential when administered in fatty acid overload conditions in HepG2 cells. We found that T. procumbens extract is a good antioxidant drug which reduces significantly lipid accumulation, suppresses fatty acid synthesis, and stimulates their oxidation. Therefore, the secondary metabolites of T. procumbens may actively prevent nonalcoholic fatty liver disease. Hence the plant is a potential source of natural antioxidant which could be useful in physiological and pathological medicine, and of great interest to food manufacturing industries. Further, detailed investigation of the active compounds of the plant for structural elucidation will contribute greatly to the development new pharmaceuticals.

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