

Phytochemicals Screening and Anti-tyrosinase Activity of Senegalese Herbal Extracts

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ABSTRACT: Several compounds and vegetable extracts are known to have an anti-tyrosinase activity. The secondary metabolites of plants contain a large number of such compounds with anti-tyrosinase properties. The chemical components sought in the selected plants are flavonoids, phenols, tannins, terpenoids, alkaloids, coumarins and sterols. This study's principal aim is to test anti-tyrosinase activity on mushroom tyrosinase for 15 extracts derived from 6 plants which grow in Senegal: *Moringa oleifera* (Lam.), *Combretum micranthum* (G. Don), *Euphorbia hirta* (L.), *Balanites aegyptiaca* (Delile), *Anacardium occidentale* (L.), *Adansonia digitata* (L.). The majority of these extracts contain all the identified families of molecules. With the exception of *B. aegyptiaca* the plants showed inhibition percentages greater than 90 % with certain extracts at 2% or 5%. The results of inhibition tests on mushroom tyrosinase for these plants have been reported for the first time in this article with the exception of *A. occidentale*.

KEYWORDS: Phytochemical test, anti-tyrosinase activity, *Moringa oleifera*, *Combretum micranthum*, *Euphorbia hirta*, *Anacardium occidentale*, *Adansonia digitata*.

I. INTRODUCTION

A. MELANOGENESIS

Skin pigmentation depends on the number, size, distribution and composition (Eu- and Phaéomélanine) of melanocytes, as well as the activity of enzymes which are involved in melanogenesis. Cutaneous pigmentation is the result of the synthesis of melanin by melanocytes and the transfer of melanosomes to keratinocytes [1]. While there are several means of inhibiting melanin synthesis, the most common cellular target for depigmenting agents is an enzyme, tyrosinase. The presence of tyrosinase is crucial for the first two oxidation reactions which occur during melanin

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synthesis: the oxidation of L-tyrosine to L-DOPA, and the oxidation of L-DOPA to L-dopaquinone. Then, two paths allow the formation of pigments:

- The dopaquinone, via 1,4-benzothiazinylalanine intermediates, leads to the formation of pheomelanin, a yellow-orange pigment responsible for light tones
- The dopaquinone, via dopachrome intermediates, and after a series of reactions, leads to the formation of eumelanin, a black-brown pigment responsible for dark tones [1, 2].

Reduction of tyrosinase activity is possible by blocking the inducing factors of tyrosinase synthesis, by inhibiting the glycosylation of tyrosinase necessary for its absorption by melanosomes, or by inhibiting the enzymatic activity of tyrosinase. The literature shows that mushroom tyrosinase is often used as a model to test inhibitors of the enzyme as it is commercially available in a pure state. However, tyrosinase isolated from mushroom is not the same as the enzyme as found in vivo. This model does not guarantee a transposition to human tyrosinase [3]. There are many compounds and vegetable extracts which enable the inhibition of the enzymatic activity of tyrosinase. The inhibition of tyrosinase is one of the existing means to regulate melanogenesis [4, 5].

B. DEPIGMENTING AGENTS

Several families of compounds contain molecules with anti-tyrosinase properties including phenols (arbutin, kojic acid, gentisic acid, hydroxycinnamic acid and their derivatives), polyphenols (quercetin, kurarinone, 2,4-resorcinol, gallic acid and its derivatives, ellagic acid, procyanidins...) [1], long-chain lipids (trilinolein, soyacerebroside I, cerebroside B), steroids (stigmast-5-en-3 β ,26-diol), triterpenoids (3 β ,21,22,23-tetrahydrocycloart-24(31),25(26)-diene, arjunilic acid...). Most monoterpenoids and diterpenoids do not have an inhibiting effect on tyrosinase. Other natural molecules ((+)-lyoniresinol) and synthetic molecules (4,4-dihydroxybiphenyl) have anti-tyrosinase properties [6]. Certain plants contain a multitude of molecules which enable the inhibition of melanogenesis. Some of these same plants contain compounds which act upon tyrosinase to inhibit its enzymatic activity, such as *cymbopogon citrates* (DC.) Stapf, *Raphanussativus* Linn or *momordicacharantia* Linn. Extracts were taken from these plants with different solvents and gave different inhibition percentages on mushroom tyrosinase [7]. In effect, the compounds extracted and the quantity extracted differed depending on the extraction solvent.

C. SELECTED PLANTS

The six plants from Senegal were selected according to their richness in compounds and families of compounds known or suspected to have an inhibitory effect against tyrosinase.

i. MORINGA OLEIFERA (LAM.)

Moringa is native to Asia and is present in most African countries, and notably Senegal. This genus that belongs to the Moringaceae family is composed of 12 species. *M. oleifera* is the best-known and most used of these species [8, 9]. The *M. oleifera* plant provides a rich combination of zeatin, quercetin, β -sitosterol, caffeoylquinic acid and kaempferol [10]. The major bioactive phenolic compounds from the leaves of *M. oleifera* are flavonoids such as quercetin and kaempferol. Methanol (80%) and ethanol (70%) proved to be the best solvents for the extraction of antioxidants from *M. oleifera* leaves [11]. *M. oleifera* leaves are rich in vitamin C, flavonoids, phenols and carotenoids [10]. *M. oleifera* seeds contain steroids and terpenoids. Nine compounds were isolated from seeds: β -sitosterol-3-O- β -D-glucopyranoside, β -sitosterol, linoleic sitosterolate, linoleic acid, 1, 2, 3-triolein, a mix of 1,3-dilinoleoyl-2-olein, 1,3-dioleoyl-2-linolein and 1,2,3-trilinolein as well as isothiocyanatomethylbenzene with ethyl chlorid as extraction solvent. [12]. Extract from *M. oleifera* seeds using ethanol gives the compounds O-ethyl-4-(α -L-rhamnosyloxy)benzyl carbamate, 4(α -L rhamnosyloxy)benzyl isothiocyanate, niazimicin, 3-O-(6'-O-oleoyl β -D-glucopyranosyl) β -sitosterol, β -sitosterol-3-O- β -D-glucopyranoside, niazirin, β -sitosterol and glycerol-1(9-octadecanoate). *M. oleifera* pods contain the compounds β -sitosterol, vitamins A, B and C, α -tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β -carotene, protein, amino acids [10].

ii. COMBRETUM MICRANTHUM (G. DON)

Kinkeliba (*Combretum micranthum*) is a shrub species of West Africa that belongs to Combretaceae family. The leaf of *C. micranthum* is used as an infusion or popular herbal tea and is an ethnomedical plant widely used in West Africa to treat numerous illnesses. It is one of the most popular teas in Senegal. The kinkeliba leaf is an important source of

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polyphenols. It contains flavonoids, catechins, organic acids, tannins... [13]. The drink's traditional preparation, using decoction, has been optimised in order to extract the maximum number of phenolic compounds. The results have shown that an optimal preparation requires the brewing of approximately 20 to 25 g of kinkeliba leaf powder in boiling water for approximately 15 to 20 minutes. The reported total phenolic compound content is 215.0 mg GAE/g [14]. The extraction of kinkeliba leaves by maceration over 72 hours with 70% ethanol shows the presence of numerous phytochemical constituents such as alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids [15]. The extraction of kinkeliba leaves with aqueous ethanol enabled identification of the following compounds: catechins, glycosylflavones, flavanes, epicatechin, gallic acid, malic acid, betaine, choline, combretine, vitexin, isovitexin (C glycosylflavone), m-inositol, sorbitol, myricetin 3O-glucoside and myricetin-3-O-rutinoside [13].

iii. *EUPHORBIA HIRTA* (L.)

The *Euphorbia hirta* plant belongs to the family of Euphorbiaceae. It is a small annual herbaceous plant common to tropical countries. The leaves of the plant are used to treat colic disorders, dysentery, coughs, asthma, worms, diarrhoea and vomiting [16]. Extraction of the leaves by maceration over 72 hours using ethanol, water and hexane as solvents enabled detection of flavonoids, tannins, polyphenols, steroids and alkaloids. The leaves of *E. hirta* contain a larger quantity of flavonoids and phenolic compounds than steroids and alkaloids [17]. The flavonoids euphorbin, leucocyanidol, camphol, quercitrin and quercitol as well as polyphenols such as gallic acid, myricitrin, 3,4-di-O-galloylquinic acid, 2,4,6-tri-Ogalloyl-Dglucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose are present in the *Euphorbia* plant [18]. Extraction of the *E. hirta* plant using acetone over one week at ambient temperature enabled the extraction of carbohydrates, proteins, lipids, flavonoids, alkaloids, saponins, resins, sterols, steroids, acidic compounds, tannins, glycosides, anthraquinones, phenols and terpenoids [19]. The *E. hirta* plant which is also used to treat skin ailments contains flavonoids (quercitrin, myricitrin...), sterols (Cycloartenol, 24-methylenecycloartenol...), tannins (euphorbin E, euphorbin A, euphorbin B, gallic acid...) and triterpenoids (α-amyrin, βamyrin, taraxerone, taxerol, β-amyrin acetate...) [20].

iv. *BALANITES AEGYPTIACA* (DELILE)

Balanites aegyptiaca is a tree native to Africa and the Middle East belonging to Zygophyllaceae family. All parts of the plant are used in traditional medicine. The plant has been used for skin illnesses, diarrhoea and jaundice [21]. This plant is present in Senegal [22]. The fruit consists of an epicarp, a mesocarp, an endocarp and a stone [23]. Aqueous extract of *B. aegyptiaca* fruit has enabled the detection of tannins, saponins, terpenoids, flavonoids, anthraquinones, alkaloids, steroids and phenols [24]. The fruit's mesocarp is completely soluble in water [25]. After maceration over 72 hours of *B. aegyptiaca* mesocarp in absolute methanol, phytochemical tests show the presence of saponins, terpenoids, phenolic compounds and alkaloids. The total quantity of phenolic compounds is 212 mg GAE/g and the total quantity of flavonoids is 11.5 mg QE/g [26].

v. *ANACARDIUM OCCIDENTALE* (L.)

Anacardium occidentale, family Anacardiaceae, is a tree native to Brazil and present in several regions of the world. Studies of a cashew variety which is present in Senegal showed the presence of quercetin-glycosides, condensed tannins and phenolic acids [27]. Vimala S. identified 5 plants which had a whitening effect on the skin, including *A. occidentale* [28]. The leaves of *A. occidentale* possess an anti-tyrosinase activity comparable to that of *Psidium guajava* and *Hibiscus tiliaceus*, which have strong skin-whitening properties [29]. Compounds from different parts of the cashew have an inhibiting activity on tyrosinase, namely anacardic acid, 2-methylcardol as well as other cardols. In addition, 2 active ingredients, 6-[8(Z),11(Z),14-pentadecatrienyl]salicylic acid and 5-[8(Z),11(Z),14-pentadecatrienyl]resorcinol, inhibit the oxidation of L-DOPA in a competitive manner [30]. Aqueous extract of the leaves of *A. occidentale* revealed the presence of phenolic compounds, flavonoids, steroids and triterpenes [31]. Tannins, phenols, flavones, flavonols, xanthenes, flavonoids, catechins and alkaloids were identified in the ethanol extract of *A. occidentale* leaves [32]. The quantity of total polyphenols detected from *A. occidentale* leaves was 37.41 and 40.26 mg GAE/mg of extract in aqueous and ethanol extract, respectively [33].

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vi. *ADANSONIA DIGITATA (L.)*

The *Adansonia* genus, Malvaceae family, includes 8 species, with one being endemic to Africa: *A. digitata* [34]. Parts of the tree are used in traditional medicine in Africa. The plant is used for the treatment of diarrhoea, malaria, and microbial infections [35]. The fruit of *A. digitata* is composed of an external shell, seeds, and pulp, representing 45%, 40% and 15% respectively. The pulp of the fruit contains citric, tartaric, malic, and succinic organic acids, and a large quantity of ascorbic acid. Other compounds are also present, sometimes in large quantity, such as carbohydrates, sugars and proteins [36]. The extraction of the epicarp of *A. digitata* fruit (baobab) with 80% methanol shows the presence of several proanthocyanidins as major compounds: (-)-Epicatechin, epicatechin-(4--> β8)-epicatechin, epicatechin-(4--> β6)- epicatechin, epicatechin-(2β--> O-->7, 4β-->8)-epicatechin and epicatechin-(4--> β8)- epicatechin-(4--> β8) epicatechin. [34]. Isopropyl alcohol extract of baobab pulp was subjected to phytochemical tests which showed the presence of carbohydrates, alkaloids, tannins, saponins, sterols, flavonoids. The pulp contains a large quantity of ascorbic acid 264.3mg/100g of pulp [37].

This study focuses on the inhibition of the enzymatic activity of tyrosinase with the aim of selecting the most promising plants among those which we have chosen.

II. RELATED WORK

The antityrosinase test is the first approach in the search for plant extracts inhibiting melanogenesis. The aim is to integrate them into the formula of cosmetic products with skin lightening effect. It is almost essential to go through this test before starting the in-vitro and in-vivo tests because they are more complex and more expensive [3, 38]. Narisa Kamkaen et al. tested the antityrosinase activity of 64 extracts derived from 16 plants (Each plant subjected to extraction with 4 different solvents) in order to develop new skin-whitening agents [39].

Whitening products have several application areas especially in cosmetics, dermatology and food. Many plants are tested for their antityrosinase activity such as *Sophora flavescens*, *Morus alba* and *Anacardium occidentale* [4, 40]. *Bellis Perennis* L. plant is known to reduce the activity of tyrosinase and is recommended to be used in skin-lightening cosmetics and in case of pigmentation disorders, hyperpigmentation or age spots [41]. Many other plant extracts are used as skin-whitening products such as *Crocus sativus* extract [42], *Licorice* extract [4], and *Japanese Angelica* extract [4]. *Artocarpus lakoocha* has been tested in vivo and has shown great promise for use in cosmetics [43].

III. MATERIALS AND METHODS

A. PREPARATION OF PLANT EXTRACTS

The various fresh plants were imported from Senegal. The plants were all air-dried in the shade for 3 weeks. They were then ground until fine and homogenous particles were obtained. The parts of the plants underwent a maceration over 72 hours in beakers. Extraction solvents used for each plant (Table 1) were selected according to the solvents used by other authors as seen above to extract the compounds of interest for this study. The maceration was undertaken at ambient temperature with agitation in beakers closed with parafilm. The extracts were filtered under vacuum with N 541 filter paper, then concentrated by rotavap.

Plants	Part of the plant	Solvent	Ratio (w : v)	Abbreviation
<i>Moringa oleifera</i>	Stems and leaves	Ethanol 70%	(1 : 5)	MOSL-Et70
	seeds	Ethanol 70%	(1 : 5)	MOSE-Et70
	Pods	Ethanol 70%	(1 : 10)	MOPO-Et70
<i>Adansonia digitata</i>	Epicarp	2-propanol 95%	(1 : 4)	ADEP-2prop
		Methanol 80%	(1 : 4)	ADEP-Me80
	Pulps and seeds	2-Propanol 95%	(1 : 2)	ADPS--2prop
		Methanol 80%	(1 : 2)	ADPS-Me80

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<i>Anacardium occidentale</i>	Leaves	Ethanol 95%	(1 : 5)	ANLE-Et95
		Water	(1 : 6)	ANLE-Aqu
<i>Balanites aegyptiaca</i>	Pulps	Methanol 80%	(1 : 1)	BAFR-Me80
		Water	(1 : 1)	BAFR-Aqu
<i>Combretum micranthum</i>	Leaves	Ethanol 70%	(1 : 8)	COLE-Et70
		Water	(1 : 8)	COLE-Aqu
<i>Euphorbia hirta</i>	All plants	Ethanol 95%	(1 : 5)	EUAP-Et95
		Acetone	(1 : 5)	EUAP-Acet

Table 1 – scientific name, part used, solvent used and ratio (w : v) of plant screened

B. PHYTOCHEMICAL SCREENING

Terpenoids: 2 mL of chloroform were added to 0.5 g of each extract. Several drops of concentrated H₂SO₄ were then added with precaution to form a layer. A reddish-brown color at the interface indicates the presence of terpenoids [24].

Flavonoids: the extracts were treated with a few drops of lead acetate solution. The formation of a yellow precipitate indicates the presence of flavonoids [44].

Tannins: approximately 0.5 g of the extract was boiled in 10 mL of water in a test tube, then filtered. A few drops of 0.1% ferric chloride were added until observation of a brownish-green or blue-black color [24].

Phenols: the test extract was extracted using ethyl acetate, and filtered with Whatman filter paper N 541. The development of a blue-black or brown coloration upon addition of 10% ferric chloride to the filtrate indicates the presence of phenol [24].

Sterols: 0.2 mL of concentrated H₂SO₄ was added to the same volume of each of the extracts in a test tube. A red color indicates the presence of sterols [24].

Alkaloids: 0.5 g of each extract was shaken with 5 mL of 1% aqueous hydrochloric acid on a vapor bath. 1 mL of filtrate was treated with a few drops of Drangedrorff's reagent. A dark-brown creamy precipitation indicates a positive test [45].

Coumarins: 1 mL of 10% sodium hydroxide was added to each 1 mL of extract. The apparition of a yellow color indicates the presence of coumarin[46].

C. ANTI-TYROSINASE TEST

Tyrosinase catalyses the formation of L-dopaquinone and dopachrome from L-Tyrosine and L-Dopa. Dopachrome is a colored compound, quantifiable by visible spectrophotometry at 475 nm. The presence of active ingredients capable of modifying enzymatic activity will result in a variation of optical density at 475 nm($\epsilon = 3600 \text{ M}^{-1} \text{ cm}^{-1}$).

Three solutions were prepared:

- Solution of L-Tyrosine at 0.5 mg mL^{-1} in HCl 0.1 M/NaOH 0.5 M (85%/15%)
- Solution of Tyrosinase at 142 UI mL^{-1}
- Solution contain plants extracts at 2% or 5%

	Witness	Blank	Assay
L - Tyrosine	1 mL	1 mL	1 mL
Buffer pH 6,8	1 mL	1 mL	---
Extract	---	1 mL	1 mL
Agitation and 5 minutes pre incubation at 37 °C			
Tyrosinase	1 mL	---	1 mL
60 minutes incubation at 37 °C			

Table 2 – Experiments conducted for the determination of the percentage inhibition of tyrosinase

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All tubes were shaken and placed in a cold water bath to stop the reaction. The absorbance of all the tubes was measured at 475 nm. Using more or less buffer does not influence the absorbance of the different tubes. The inhibition percentage was calculated by the formula given below [38]:

$$\% \text{ tyrosinase inhibition} = \frac{A_{\text{witness}} - (A_{\text{assay}} - A_{\text{blank}})}{A_{\text{witness}}} \times 100$$

IV. RESULTS AND DISCUSSION

Firstly, phytochemical tests were undertaken for the detection of 7 secondary metabolites (table 3) in order to determine the chemical composition of each extract.

Secondary metabolites are molecules which do not intervene directly in plant growth. These molecules are the origin of the active ingredients found in medicinal plants. With a few exception, the extracts of the selected plants contain flavonoids, phenols, tannins, terpenoids, alkaloids, coumarins and sterols. Epicarp of *A. digitata* does not contain alkaloids and coumarins. The presence of flavonoids and phenols was not detected in extracts of pulps and seeds of *A. digitata*. Extracts of *B. aegyptiaca* show an absence of tannins. All the secondary metabolites were identified in 95% ethanol extract of *E. hirta*, while acetone extraction is marked by the absence of terpenoids, alkaloids and sterols.

Abbreviation (plants)	Secondary metabolites						
	FLA	PHE	TAN	TER	ALC	COU	STE
MOSL-Et70	+	+	+	+	+	+	+
MOSE-Et70	+	+	+	+	+	+	+
MOPO-Et70	+	+	+	+	+	+	+
ADEP-2prop	+	+	+	+	-	-	+
ADEP-Me80	+	+	+	+	-	-	+
ADPS--2prop	-	-	+	+	+	+	+
ADPS-Me80	-	-	+	+	-	+	+
ANLE-Et95	+	+	+	+	+	+	+
ANLE-Aqu	+	+	+	+	+	+	+
BAFR-Me80	+	+	-	+	+	+	+
BAFR-Aqu	+	+	-	+	+	+	+
COLE-Et70	+	+	+	+	+	+	+
COLE-Aqu	+	+	+	+	+	+	+
EUAP-Et95	+	+	+	+	+	+	+
EUAP-Acet	+	+	+	-	-	+	-

Table 3 – Result of the phytochemical screening of the plants

FLA : flavonoids ; PHE : phenols ; TAN : tannins ; TER : terpenoids ; ALC : alkaloids ; COU : coumarins ; STE : sterols ; (+) : presence ; (-) : absence.

The anti-tyrosinase activity of each vegetable extract on mushroom tyrosinase was then determined using L-tyrosine as substrate. The results of the anti-tyrosinase activity expressed as an inhibition percentage of the 15 plant extracts are shown in table 4.

Among the 6 plants selected, 5 give tyrosinase inhibition percentages greater than 90 %: *M. oleifera*, *A. digitata*, *A. occidentale*, *C. micranthum* and *E. hirta*.

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Aqueous extract and 80% methanol extract of *B. aegyptiaca* shows inhibition percentages of only 32.3 % and 70.9 %, respectively. The leaves of *A. occidentale* give an inhibition percentage of 98.6 % with ethanol while the aqueous extract only inhibits tyrosinase by 63.9 %. In effect, the extraction solvent has a large influence on the extraction of tyrosinase-inhibiting compounds and on the quantity extracted according to their solubility in the extraction solvent. *M. oleifera* seeds give a high inhibition percentage (92.8%) compared to other parts of the same plant which gives lower inhibition percentages. Other plants present very high inhibition percentages (>90%) with certain extraction solvents. In this study, plants with extracts giving inhibition percentages greater than 90% with an extract solution of at least 5% are considered to be very promising.

Abbreviation (plants)	Plant extract at (%)	Inhibition percentage (%)
MOSL-Et70	5%	65
MOSE-Et70	5%	92.8
MOPO-Et70	5%	52.6
ADEP-2prop	5%	77
ADEP-Me80	5%	88.5
ADPS--2prop	5%	91.2
ADPS-Me80	5%	74.2
ANLE-Et95	2%	98.6
ANLE-Aqu	5%	63.9
BAFR-Me80	5%	70.9
BAFR-Aqu	5%	32.3
COLE-Et70	5%	94.7
COLE-Aqu	5%	91.3
EUAP-Et95	2%	96.9
EUAP-Acet	2%	93.6

Table 4 – Anti-tyrosinase activity of the 15 plant extracts expressed as an inhibition percentage

The families of compounds previously identified in phytochemical tests contain compounds capable of inhibiting tyrosinase. These plants contain a large variety of secondary metabolites which gives them medicinal properties known in traditional African medicine. We suspect that polyphenols, flavonoids, terpenoids and sterols are responsible for the anti-tyrosinase activity found in the extracts of these plants. The extracts were only tested for their anti-tyrosinase properties. However, the richness of their chemical constituents may inhibit melanogenesis by acting on other inhibition sites. More advanced biological tests on human melanocyte cultures are necessary to confirm the inhibiting activity of these plant extracts on melanogenesis. These plants could be utilized for the preparation of cosmetic products with skin lightening effects.

V. CONCLUSION

Phytochemical tests for the detection of secondary metabolites has enabled the identification of flavonoids, phenols, tannins, terpenoids, alkaloids, coumarins and sterols. The results obtained of the inhibition percentages on mushroom tyrosinase are very promising. The extracts of *M. oleifera* seeds, *A. digitata* pulps and seeds, *A. occidentale* leaves, *C. micranthum* leaves and all parts of the plant *E. hirta* enabled over 90% inhibition of mushroom tyrosinase. This study has enabled the selection of plants which are potentially useable as depigmentation agents in plant-based cosmetic products. Further studies are necessary in order to confirm the lightening effect of these plant extracts on skin.

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