ORIGINAL ARTICLE

In-silico Prediction Analysis of Polyphenolic Contents of Ethanolic Extract of *Moringa oleifera* Leaves

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ABSTRACT

Introduction: *Moringa oleifera* is widely consumed as a source of nutrients and as traditional medicine for treating myriads of diseases in Southeast Asia, Africa, and South America. Polyphenols are abundantly found in the leaves of *Moringa oleifera*, particularly astragalin and quercetin, and have shown antioxidant and anti-inflammatory activities. In the present study, the polyphenols in *Moringa oleifera* leaves were identified and quantified, followed by *in-silico* pharmacokinetics prediction. **Methods:** *Moringa oleifera* ethanolic leaf extract (MOEE) was prepared by macerating dry powdered leaves of *Moringa oleifera* in 70% ethanol, then subjected to ultra-high-performance liquid chromatography-mass spectrometry (UPHLC-MS/MS) to identify polyphenols and quantification by HPLC. *In-silico* pharmacokinetic prediction analysis of the polyphenols was performed using the SwissADME web tool. **Results:** Eight polyphenols were identified, among which astragalin and quercetin were quantified. *In-silico* prediction analysis using SwissADME demonstrates the drug-likeness of the polyphenols in MOEE based on their physicochemical and ADME (absorption, distribution, metabolism and excretion) properties. MOEE contains polyphenols that can serve as lead compound to developing drugs, but only some are predicted to have high GI absorption index demonstrating their utility in oral medication drug development. Moreover, all the polyphenols determined in this study are predicted to be non-brain permeant, demonstrating their limited utility in developing drugs that target brain-related pathologies. **Conclusion:** Eight polyphenols were identified by UHPLC/MS analysis of *Moringa oleifera* leaves. Their ADME pharmacokinetics properties predicted some of the polyphenols as favourable candidates for drug development and lead optimisation, thus positioning the *Moringa oleifera* as an important source of small molecules for drug development.
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Keywords: *Moringa oleifera*; Polyphenols; *In-silico* ADME prediction analysis; Pharmacokinetics; Computer-aided drug discovery

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INTRODUCTION

According to the WHO data, over 60%-80% of the world's population depends on traditional, complementary, and integrative medicine, which clearly underscores traditional medicine's role in modern health care. [1, 2]. Plants rich in polyphenols possess antioxidant and anti-inflammatory properties and have been demonstrated to alleviate myriads of diseases [3]. One of the plants enriched with polyphenols is *Moringa oleifera*. *Moringa oleifera*, known in Malaysia as 'sayur kelor' and in Tamil as 'Murunggai', is consumed as a source of nutrients and for medicinal purposes [4, 5].

Moringa oleifera is a small, medium-sized tree in the Moringaceae family, genus: Moringa, and the most investigated species in the genus, consisting of 13 species [5, 6]. *Moringa oleifera* is widely cultivated in East and Southeast Asia, Africa, Latin America, the Caribbean, and the Pacific Islands, with about 36 variants [4, 7, 8]. *Moringa oleifera* plant contains phytocompounds with medical properties; hence each part of the plant

has a medicinal use that was claimed to cure at least 300 ailments in Ayurveda medicine [9-12]. Specifically, the polyphenols—astragalin and quercetin— have been reported to be abundantly found in the leaves of *Moringa oleifera* [13, 14]. By far, the leaves of *Moringa oleifera* (Fig 1) are the most investigated compared with other parts of the plant [3, 4, 15]. Thus, this work investigates the polyphenolic contents of ethanolic leaf extract of *Moringa oleifera* (MOEE) using ultra-high-performance liquid chromatography-mass spectrometry (UPHLC-MS/MS) and high-performance liquid chromatography (HPLC), followed by *in-silico* pharmacokinetics prediction analysis to determine the drug-likeness and lead-likeness potentials of the polyphenols providing information for the medicinal chemist to support their drug discovery effort using *Moringa oleifera* plant.

Fig. 1 : Fresh *Moringa oleifera* (lam.) leaves. Image captured by the investigator.

MATERIALS AND METHODS

Source of *Moringa oleifera* leaves and UHPLC-MS/MS analysis

Fresh and healthy leaves of *Moringa oleifera* were obtained from a herbal farm located at Kampar, Perak. The leaves were processed immediately at the Ethno Resources Sdn Bhd., Sungai Buloh, Selangor, Malaysia. The leaves were identified and authenticated at the Institute for Medical Research (IMR) and Universiti Putra Malaysia (UPM). The leaves were then processed immediately at the Ethno Resources Sdn Bhd., Sungai Buloh, Selangor. Briefly, the leaves were washed several times in tap water, followed by two washes with distilled water and allow to dry overnight. The dry leaves were oven-dried at 40°C for 72 hours to crispy texture, then ground to powder approximately 1 mm sieve size. The powdered leaves were tested against microbes, and heavy metals. The leaves powder was then extracted by maceration method using 70% ethanol. The leaves powder where mixed with 70% ethanol at a ratio of 1:20 (w/v), 10 grams of the powdered leaves where mixed with 200 mL of 70% ethanol. The mixture was homogenized and allow to stand for 72 hours at room temperature with intermittent shaking. Then, the mixture was filtered with a Whatman filter No. 1 paper. Thereafter, ethanol was evaporated from the filtrate using a rotary evaporator and frozen at -80°C refrigerator. Lastly, the filtrate was freeze dried (-40°C and 1.554 Pa) in a freeze dryer to form a dry concentrate that was stored at -20°C refrigerator use. UHPLC was performed by a Thermo Fisher system fitted into a Dionex Ultimate 3000 UHPLC coupled with a diode-array DAD-3000 detector, maintaining the column compartment at 40°C and separated by C_{18} reversed-phase Acquity UPLC BEH C_{18} 1.7 mm (2.1 x 100 mm) with a guard column. Followed by MS analysis on a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap mass spectrometer, with heat electrospray ionisation (HESI) performed in both positive and negative modes, with a negative and positive spray voltage of 4.0 and 3.7 kV respectively. The LC-MS data were analysed using Thermo Xcalibur 2.2 SP1.48 (Thermo Fisher Inc. USA). Compounds were identified by Compound discoverer 2.1 (Thermo Fisher inc. USA).

High-Performance Liquid Chromatography (HPLC) analysis of 70% ethanolic leaves extract of *Moringa oleifera* (MOEE)

The HPLC instrument used was Waters Alliance 2695 (USA) with a 996 Photodiode Array detector liquid chromatograph fitted with a C-18 reverse-phase (particle size, 4 mm) column (250 mm 4 mm i.d.) (Phenomenex Synergi Polar-RP, USA). For quantification of the standard compounds in 70% ethanolic dry powdered leaves extract of *Moringa oleifera* (Quercetin 3-O-glucoside, and Kaempferol 3-O-glucoside) were 0.005% trifluoroacetate in water (solvent A) and 0.005% trifluoroacetate in acetonitrile (solvent B) were used as mobile phase, when 10 µL of th extract was injected into the HPLC system. The solvent gradients involves 95% A for 3 minutes, 60% solvent A in 43 minutes, 20% solvent B in 3 minutes, 52% solvent A in 3 minutes, 95% solvent A until the completion of the run at 55 minutes. The flow rate of the mobile phase is constant at 0.8 mL per minutes and the phenolics compounds in the eluates were detected with ultraviolet dual-array detectors (Water Alliance 2695, USA) set at 265.1 and 255.6 nm. Instrument control and data handling was performed using the Waters Empower 2 software for windows [14, 16-18].

In-silico pharmacokinetics predictions

The free web tool SwissADME was used to evaluate the pharmacokinetics— absorption, distribution,

metabolism, excretion (ADME)—, drug-likeness, and medicinal friendliness of small molecules. One of the advantages of SwissADME is the evaluation of several parameters in each of the ADME factors to enable a holistic and robust choice. Important descriptors to access the potential of a molecule to be an effective drug lead include bioavailability indices, physicochemical properties, lipophilicity, water solubility and pharmacokinetic indices displayed in the output to enable informed decisions by drug developers. For example, in the evaluation of drug-likeness, not only Lipinski (Pfizer) rule of five is accessed, but also The Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer) were equally evaluated for a comprehensive evaluation of drug-likeness. These are also true for the rest of the parameters appraised by SwissADME [19]. The submission page of the SwissADME is accessed from a web browser (http://www.swissadme.ch). The imput zone consist of a molecular sketcher that is based on ChemAxon's Marvin JS (https://www.chemaxon.com), which enables the imput of chemical structure file from external database, drawing, and editing of 2D chemical structure of a compound. Subsequently, the sketch is transferred to a list of molecules search box, which is the actual input for computation that convert the sketch to SMILES string. The list contain one molecule per line defined by a SMILES and optionally separated by a space. The computation display result in 1 to 5 seconds for drug-like chemical compounds.

RESULTS

Determination of polyphenolic contents of MOEE by UHPLC-MS/MS and HPLC

Fig. 2 shows the UHPLC/MS chromatogram analysis of the MOEE extract. The eight phenolics with the highest peak are chlorogenic acid, quercetin-3b-D-glucose, astragalin, quercetin, luteolin, hispidulin, quercitrin, isorhamnetin, and vitexin. Most of the polyphenols were detected in the negative ion mode of the UHPLC-MS/MS than in the positive mode. They were all eluted at a retention time between 4- and 17-min. Separation and quantification of the two major polyphenolics, astragalin and quercetin, by HPLC yielded a percent content of 0.0698±0.0006% w/w of astragalin and 0.0084±0.0002 of quercetin % w/w in 1 mg/mL of dried leaves ethanolic extract of *Moringa oleifera* (Table I).

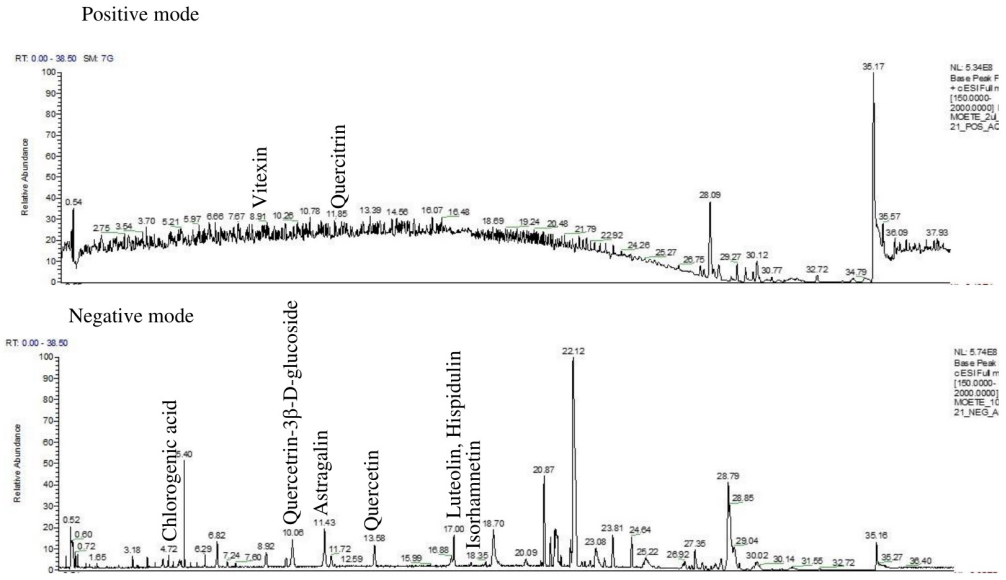

Fig. 2 : UHPLC-MS chromatogram of MOEE. Eight polyphenols were identified in the MOEE sample: astragalin, quercetin, vitexin, chlorogenic acid, quercetin 3b-D glucoside, luteolin, hispidulin, and isorhamnetin. Retention time between 4- and 17-min.

Table I : Quantification of MOEE Marker Compounds by HPLC

	Sample	Retention time (min)	Purity Threshold	Area (UV×—sec)	Amount (mg/mL)
Astragalin	Standard	32	1.467	5949305	
	M. oleifera	32.6	39.729	2139641.3	0.0698
	±Std Dev	±0.023	±0.831	±3829.9	±0.0006
Quercetin	Standard	42	1.183	9629611.0	
	M. oleifera	41.9	88.488	403128.0	0.0084
	± SD	±0.11	±3.532	±19748.4	±0.0002

Regression equation:

Astragalin, y = 6.16e + 0.06x Quercetin, y = 9.63e + 0.06x. Where y = peak area (UV×sec), x = concentration of astragalin or quercetin in mg/mL.

***In silico* ADME prediction by SwissADME tool**

Table II shows the result of the web-based tool SwissADME on physicochemical properties: lipophilicity, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of the polyphenols detected by UHPLC in MOEE. The *in-silico* prediction analysis demonstrates that the polyphenols have lipophilicity, molecular weight and water solubility within the acceptable range of (-0.7 to +5.0), (150 to 500 g/ml), and (≤6), respectively. On the other hand, they exhibit various polarity index; hispidulin, isorhamnetin, luteolin, vitexin, and quercetin, have polarity within the recommended range of (20 to 130 $Å^2$), while astragalin, chlorogenic acid, quercitrin, and quercetin-3b-D-glucoside demonstrate polarity outside the recommended range (>130$Å^2$), which translate to their high and low GI absorption rate respectively. Further, except for quercetin, hispidulin, Isorhamnetin, and luteolin, the remaining polyphenols are predicted to have a carbon saturation in the Csp3 within the acceptable value of (≥0.25). In addition, the number of rotatable bonds, a measure of the flexibility of a compound, is also within the acceptable value of (≤9) in all the polyphenols.

Lastly, the result of boiled egg prediction analysis (Fig. 3) evaluates the passive and/or active gastrointestinal absorption (HIA) and brain penetration (BBB) of a molecule based on prediction analysis of WLOGP (lipophilicity)-versus-TPSA (polarity). Hispidulin, isorhamnetin, luteolin, and quercetin are predicted as well absorbed through the gastrointestinal tract but not brain permeant. Conversely, quercitrin, vitexin, chlorogenic acid, and astragalin are predicted

Table II : *In silico* Analysis of MOEE polyphenols

Compound ADME properties	Astragalin	Quercetin	Chlorogenic acid	Hispidulin	Quercitrin	Isorhamnetin	Luteolin	Quercetin 3 b-D-glucoside	Vitexin
Lipophilicity (XLOGP3)	0.72	1.53	-0.42	2.99	0.86	1.87	2.53	0.36	0.21
Size (MW):	448.38	302.24	354.31	300.26	448.38	316.26	286.24	464.38	432.38
Polarity (TPSA):	190.28	131.36	190.28	100.13	190.28	120.36	111.13	210.51	181.05
Solubility (Log S)	-3.18	-3.16	-1.62	-3.99	-3.33	-3.36	-3.71	-3.04	-2.84
Water solubility	Soluble	Soluble	Very soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
Saturation (Fraction of carbons in Csp3)	0.29	0.00	0.38	0.06	0.29	0.06	0.00	0.29	0.29
Number rotatable	4	1	5	2	3	2	1	4	3
GI absorption	Low	High	Low	High	Low	High	High	Low	Low
BBB permeant	No	No	No	No	No	No	No	No	No
P-gp substrate	No	No	No	No	No	No	No	No	No
Drug-likeness (Lipinski rule)	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Drug-likeness (Ghose rule)	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
Bioavailability score	0.17	0.55	0.11	0.55	0.17	0.55	0.55	0.17	0.55
Lead-likeness	No	Yes	No	Yes	No	Yes	Yes	No	No
Synthetic accessibility (1–10)	5.29	3.23	4.16	3.12	5.28	3.26	3.02	5.32	5.12

MW: molecular weight; GI: gastrointestinal; BBB: blood-brain barrier; P-gp: Substrate glycoprotein.

Lipophilicity: XLOP3 between -0.7 and +5.0; Size: MW between 150 and 500 g/mol; Polarity: TPSA between 20 and 130 E^2; Solubility: Log S ≤ 6; Saturation: fraction of carbons in the sp3 hybridization ≥ 0.25; Flexibility: ≤ 9 rotatable bonds. Synthetic accessibility: 1 (very easy) to (10) very difficult to synthesize. The polyphenols are not orally bioavailable, because too polar, with the exception of quercetin, hispidulin, isorhamnetin, luteolin, and vitexin.

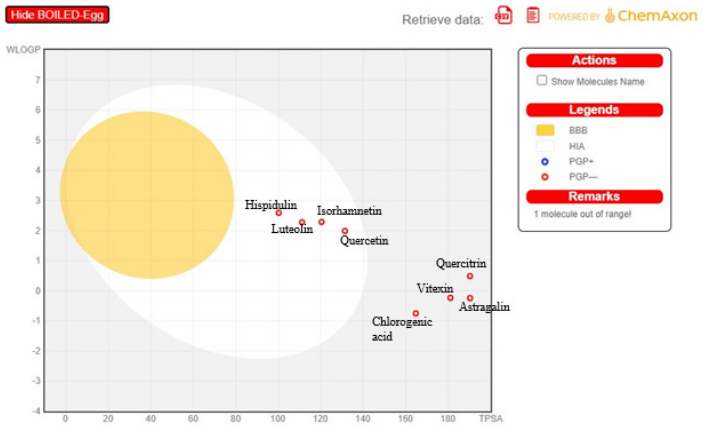

Fig. 3 : The Boiled Egg Model of Moringa oleifera Leaves Polyphenols. Evaluate the passive and/or active gastrointestinal absorption (HIA) and brain penetration (BBB) of a molecule based on predicted values of WLOGP (lipophilicity) and TPSA (polarity). The White region denotes a high probability of passive absorption through the gastrointestinal tract. The yellow region (yolk) denotes a high probability of brain penetration. The yolk and the white regions are not mutually exclusive. The coloured point on the compound, if it is blue, predicts that the compound is actively effluxed by P-gp (PGP+), and if it is red is predicted as a non-substrate of P-gp (PGP-). Astragalin, Chlorogenic acid, vitexin, and quercitrin are predicted as not absorbed and not brain penetrant (outside the Egg) and PGP- (red dot). Quercetin, hispidulin, luteolin, and isorhamnetin are predicted as well absorbed but not accessing the brain (in the white area) and PGP- (red dot). quercetin-3b-D-glucoside is predicted not absorbed and not BBB penetrant due to values outside the range of the plot (WLOGP -0.54 and a TPSA of 210.51 E2).

as not passively absorbed through the gastrointestinal tract and not brain penetrant. Quercetin 3-b-D-glucoside is predicted as not absorbed by the gastrointestinal tract and not brain permeant because of values outside the range of the plot (WLOGP -0.54 and a TPSA of 210.51 $Å^2$). All 8 polyphenols are not substrate for substrate glycoprotein (P-gp); therefore, they are not pumped back into the gastrointestinal lumen once absorbed.

DISCUSSION

The ethanolic leaf extracts of the *Moringa oleifera* (MOEE) plant have abundant polyphenols and hence become relevant in identifying plants with antioxidant and anti-inflammatory properties [14, 20, 21]. Polyphenols are classified and grouped based on the number of phenolic rings and the elements to which they bind. Polyphenols are divided into flavonoids and non-flavonoids. Flavonoids include six subgroups of compounds, flavonols, flavones, flavanols, isoflavones and anthocyanins, while the non-flavonoids include lignans, tannins, stilbenes and phenolic acid [22]. In the present study, UHPLC analysis detected the presence of top 8 polyphenolics in the ethanolic extract of *Moringa oleifera* (MOEE) as astragalin, quercetin, vitexin, chlorogenic acid, quercetin 3β-D glucoside, luteolin, hispidulin, and isorhamnetin, that exhibited antioxidant and anti-inflammatory activities [23-25]. It thus may explain the potency of the *Moringa oleifera* plant in treating various diseases. Quantitative estimation of the two abundant polyphenols, flavonol (quercetin) and astragalin (trihydroxyflavone) in the extract (1 mg/mL) were 0.0084±0.0002 and 0.0698±0.000 % w/w respectively. Although this finding corroborates with previous findings on the enrichment of 70% ethanolic extract of *Moringa oleifera* with quercetin and astragalin, their values obtained are slighter higher than that of the present study [13]. Differences in geographical locations and processing methods might contribute to the variation in the amount of the analysed phytoconstituents. These polyphenols can be regarded as important compounds that protect against diseases.

This is the first study to describe the physicochemical and ADME properties of MOEE polyphenols using *in-silico* analysis. Poor pharmacokinetic profiles (absorption, metabolism, distribution, and excretion) are one of the main causes of drug failure during clinical phase trials on drug development [26]. Interestingly, computer models (*in-silico*) provide valid alternatives for predicting ADME during the initial phases of drug development [27]. Further, access to and concentration of drugs at therapeutic targets enhances their biological activity and reduces their toxicity profile, which can also be predicted by computer modelling. *In-silico* models utilise molecular structure to determine the drug's oral bioavailability (28) and substructures to ascertain compounds that are most likely unstable, reactive, toxic, and susceptible to interfering with biological reactions [28]. The physicochemical properties of the polyphenols outlined in the result section predicted that; quercetin, hispidulin, isorhamnetin, and luteolin are predicted to be readily absorbed through the gastrointestinal tract, while astragalin, chlorogenic acid, quercitrin, quercetin-3b-D glucoside, and vitexin are predicted to be unabsorbed by the gastrointestinal tract.

Moreover, all the polyphenols determined in this study have the inability to cross the blood-brain barrier demonstrating their limited utility in developing drugs that target brain-related pathologies. However, using the Qikprop software Zolkiffly et al. (2021) demonstrate the QPlogBB (serving as brain blood partition coefficient) of vitexin to be within the acceptable range for brain permeability and proposes its utility in developing Alzheimer's disease drugs [2]. This difference might be because of the different datasets in the software algorithm used in the prediction, which has a different reliability index;

however, further *in-silico* evaluation is needed to resolve this difference. Interestingly, all the polyphenols are not substrate for the substrate glycoprotein (P-gp)—a member of the ATP-binding cassette transporters— that are involved in the active efflux of small molecules across biological membranes, for instance, from the gastrointestinal wall to the lumen or from the brain. Thus, it is of great importance for drug discovery to predict the susceptibility of small molecules to be a substrate for P-gp.

Further, the drug-likeness, which assesses the chance for a molecule to become an oral drug candidate concerning its bioavailability, was also evaluated. Except for astragalin and quercetin-3β-D-glucoside, the remaining polyphenols are predicted to have a good drug-likeness due to their high predicted bioavailability index (17% to 55%),

Lastly, astragalin, chlorogenic acid, quercitrin, and quercen-3b-D-glucoside are predicted not suitable as lead drugs for optimisation to a more potent drug due to their molecular weight greater than 350 g/ml. In contrast, quercetin, hispidulin, isorhamnetin, luteolin, and vitexin are predicted as suitable for lead optimisation because of molecular weight less than 350 g/ml based on the SwissADME guidelines. Similarly, their synthetic accessibility (SA), which is ranked from 1 to 10, with 1 being very easy to synthesise and 10 being very difficult to synthesise, helps in the virtual screening of molecules that can be easily synthesised and subjected to biological assays or other experimental procedures. The SA depends on the molecule's size and complexity (such as chiral centres, macrocycles and spiro functions). Here also, in concordance with their lead-likeness results, astragalin, quercitrin, quercetin-3b-D-glucoside, and vitexin are predicted to be moderately challenging to synthesise, while quercetin, hispidulin, isorhamnetin, luteolin, and chlorogenic acid are expected to be easy to synthesise. A caveat to this study is the absence of *in-silico* prediction analysis on network pharmacology and molecular docking of the potential cellular targets of these polyphenols that will help in elucidating their underlying mechanism of action; however, such prediction analysis of *Moringa oleifera* leaves polyphenols on periodontitis has been reported [21].

CONCLUSION

In conclusion, this study demonstrates that the ethanolic extract of MOEE contains abundant polyphenols. Their ADME pharmacokinetics properties predicted some of the polyphenols as favourable candidates for drug development and lead optimisation, thus positioning the *Moringa oleifera* as an important medicinal plant with small molecules that can be utilised for drug development. The small molecules identified in *Moringa oleifera* leaves undoubtedly posit the plant as a potential commercial venture.

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