EVALUATION OF MYANMAR THANAKHA (*Hesperethusa Crenulata* (Roxb.) M. Roem) AND PROCESSING OF ITS

Dr. Nwe Nwe Aung
Department of Industrial Chemistry
Yadanabon University, Mandalay, Myanmar

ABSTRACT

Thanakha, grown abundantly in central Myanmar, is well-known as a traditional, natural and organic skin care herbal material which is adored and widely used by the Myanmar society since centuries ago. All parts of this plant are useful as indigenous medicine, cosmetics and perfumery. Application of Thanakha enhances beauty, acts as perfume and has sun protection, astringent and antiseptic properties. Thanakha powder, one of the ecofriendly products, is 100% organic since the barks and roots are simply thoroughly ground. The main aim of research was to study the characteristics of Thanakha by preliminary phytochemical test, anti-microbial activity of different solvent extract and processing of its natural products. Thanakha samples were collected from Sagaing Region and Magwe Region. Different solvent extracts from Thanakha showed anti-microbial activities against certain microorganisms. From the point of view of health hazard, evaluation of the trace elements in Thanakha is also important factor. The contents of elements in Thanakha were determined by Energy Dispersive X-ray Fluorescence Technique (EDXRF). Surface morphology of Thanakha powder was also studied by SEM. This research shows that Myanmar Thanakha is an anti-bacterial/ anti-fungal. In addition, the presence of toxic heavy metals like mercury and arsenic were not found in most of the Thanakha samples and thus toxic pollutants cannot be occurred.

KEYWORDS

Thanakha, herbal material, anti-microbial, trace elements, surface morphology

INTRODUCTION

Thanakha is well-known in Myanmar as a kind of make-up which is adored and widely used by the Myanmar women of all ages since over 2000 years ago. The fragrance of Thanakha stimulates the mind to be fresh and clear and imparts the beauty of face and body and smoothness of skin in the form of soft and wet texture by continuous use. Most Myanmar women use branches,

stems and roots of Thanakha plant (U Tha H la, 1974). T hanakha, well-known in M yanmar, belongs t o t he family " *Rutaceae*" a nd g enus "*Hesperethusa* ".

OBJECTIVES OF THE STUDTY

To study the characteristics of Thanakha by pr eliminary phy to-chemical t est, an timicrobial a ctivity of di fferent s olvent e xtract a nd processing of its natural products.

MATERIALS AND METHODS

Sample Collection

Fresh s amples o f Thanakha were collected f rom S hinmataung, Yesagyo Township, P akokku T own-ship, Magwe R egion a nd S hwebo Township, Sagaing Region(Figure 1).

Sample Preparation

Different E xtracts o f T hanakha samples from S hinmataung w ere prepared by us ing di fferent s olvents for phy tochemical a nd anti-microbial activity. T hanakha s amples co llected from Yesagyo T ownship, P akokku Township, Magwe R egion a nd Shwebo T ownship, S againg R egion were collected , air-dried and the bark was removed from the stem. The bark was chipped into very small pieces of approximately 1 c ubic c entimeter in size a nd g round a nd pa ssed through 200 mesh, 300 m esh a nd 400 mesh screens. T he p owder s amples w ere stored in a n a ir-tight c ontainer f or determining trace elements.

Phytochemical Analysis

Solvent extracts of Shinmataung Thanakha w ere t ested t o r eveal the presence of a lkaloids, g lycosides, saponin glycosides, steroids, phenolic compounds, flavonoids and tannin.

Anti-microbial Activity

25 g of powder from stem and root of S hinmataung T hanakha w as filled in a th imble a nd e xtracted respectively w ith n -hexane, be nzene, ethyl acet ate, ethanol an d special boiling poi nt s olvent (SBP) us ing a Soxhlet extractor. A ll th e e xtracts were s ubjected t o an ti-microbial activity a ssay. The s ensitivity testing of the extracts were determined using agar-well di ffusion m ethod. The following microorganisms were used: *Bacillus pumalis, Bacillus subtilis, Candida albicans, Mycobacterium species, Pseudomonas aerguinosa and Staphylococcus aureus.*

Determination of Trace Elements

The T hanakha pow der of various r egions w ere analyzed for trace el ements co ntent using E nergy Dispersive X -ray F luorescence T ech-nique.

SEM Studies

The microstructure of Thanakha powder of va rious r egions were analyzed us ing S EM (Scanning Electron M icroscopy) JSM -6510 JEOL, Japan.

RESULTS AND DISCUSSION

Phytochemical analysis of solvent extracts of Shinmataung Thanakha (Table1) revealed the presence of alkaloids, glycosides, saponin glycosides, steroids, phenolic compounds, flavonoids and tannins and absence of reducing sugars. Alkaloids, saponins and tannins are to be biologically active. Due to the presence of alkaloids in Thanakha plant, it gives a slight bitter taste.This analysis showed that the active principle responsible for the anti-microbial activity was a phenolic compound. From the results of the phytochemical analysis, Thanakha could be used to formulate new and more potent anti-microbial drugs of natural origin (Table 1).

Biological screening is a useful tool for screening pharmacological activity. Agar well diffusion method is simple, economical and reproducible and is quick to perform. *In vitro* study is the fundamental and essential for the development of the natural product. From the results of Table (2), (3), (4) and Figure (2) and (3), anti-microbial activity assay of all the five extracts revealed that ethyl acetate and benzene extract showed significant activity against all organisms. Other solvent extracts viz., n-hexane, ethanol, and SBP did not show significant zone of inhibition for some organisms. However, ethanol extract of 3 stem bark inhibited 5 out of 6 tested organisms and all ethanol extracts of the whole Thankha plant exhibited against *Mycobacterium* which are found in skin lesions and leprosy. In addition, n-hexane extract of root bark showed significant inhibition zones against *B.substilis*, *S-aureus* and *Mycobacterium species*. According to this screening test, various parts of Thanakha were found to possess anti- microbial activity.

In Myanmar, Thanakha is used not only as cosmetics but also as indigenous medicine. From the point of view of health hazard, it is better to avoid the use of cosmetics containing toxic elements. According to these facts, evaluation of the trace elements in Thanakha is also an important factor (Table 2, 3, 4 and Figure 2, 3).

Trace elements are indication of the presence of non-volatile inorganic compounds. The contents of elements in Thanakha were determined by Energy Dispersive X-ray Fluorescence Technique (EDXRF). According to the EDXRF results in Table (5) and its relevant EDXRF graph (Figure 4) , it is found that the contents of calcium, iron and potassium were relatively higher than those of the other minerals. The presence of toxic heavy metals like mercury and arsenic were not found in Thanakha from different regions (Table 5 and Figure 4).

By using SEM to study the surface morphology of Thankha powder (Figure 5), the powder which had a particle size (200 mesh) could be seen to contain a lot of fibre. For the preparation of Thanakha products, the particle size of

Thanakha powder plays a significant part in determining formulation elegance, rate of settling, absence of caking and final stability of the product. Therefore, Thanakha powder having particle size (300mesh) should be used to increase the adhesive power and reduce the abrasion of the skin (Figure 5).

Table 1. Phytochemical Investigation of Thanakha.

No	Test	Solvent	Reagent	Observation		Result	
				Stem	Root	Stem	Root
1	Alkaloids	5% HCl	Mayer's reagent	White precipitate	White precipitate	+	+
2	Glycosides	H_2O	10% Lead acetate solution	precipitate	precipitate	+	+
3	Reducing sugars	H_2O	Fehling'solution	No precipitate	No precipitate	_	_
4	Tannins	H_2O	1% Ferric chloride solution	precipitate	precipitate	+	+
5	Steriods	Pet. ether	Acetic anhydride and conc. Sulphuric acid	Green colour	Green colour	+	+
6	Saponins	H_2O	Distilled water	Frothing	Frothing	+	+
7	Flavonoids	EtOH	Mg ribbon & conc. HCl	Pink colour	Pink colour	+	+
8	Phenolic Group	H_2O	Ferric chloride solution	Violet color	Violet color	+	+

(+) Present (-) Absent
Source: Original Research

Table 2. Microorganisms and Their Respective Code Number used for Antimicrobial Activity.

No.	Organisms	Code No.
1	*Bacillus pumalis*	IFO-12102
2	*Bacillus subtilis*	ap-0225015
3	*Candida albicans*	IFO-1060
4	*Mycobacterium species*	IFO-3158
5	*Pseudomonas aeruginosa*	IFO-3080
6	*Staphylococus aureus*	ATCC-12877

IFO : Institute of Fermentation, Osaka, Japan
ATCC : American Type Culture Collection
Source: Development Centre for Pharmaceutical Technology, Ministry of Industry.

Table 3. Anti-microbial Activity of Different Solvent Extracts of Thanakha Stem.

		Organisms					
Sample	**Solvents**	***B.subtilis***	***S.aureus***	***P.aeruginosa***	***B.pumalis***	***C.albicans***	***M.spscies***
Stem	n-hexane	-	-	-	-	-	-
	benzene	13mm (+)	12mm (+)	14mm (+)	14mm (+)	14mm (+)	17mm (+)
	ethyl acetate	33mm (+++)	24mm (+++)	32mm (+++)	31mm (+++)	37mm (+++)	29mm (+++)
	Ethyl alcohol	-	-	-	-	-	12mm (+)
	SBP	-	-	-	-	-	12mm (+)

Agar well- 10mm
10mm~14mm (+)
15mm~19mm (++)
20mm above (+++)
Source: Original Research

Table 4. Anti-microbial Activity of Different Solvent Extracts of Thanakha Root.

		Organisms					
Sample	**Solvents**	***B.subtilis***	***S.aureus***	***P.aeruginosa***	***B.pumalis***	***C.albicans***	***M.spscies***
Root	n-hexane	17mm (+)	12mm (+)	-	-	-	12mm (+)
	benzene	15mm (+)	15mm (+)	14mm (+)	12mm (+)	14mm (+)	15mm (+)
	ethyl acetate	19mm (++)	25mm (+++)	20mm (+++)	20mm (+++)	20mm (+++)	23mm (+++)
	Ethyl alcohol	-	-	-	12mm (+)	14mm (+)	14mm (+)
	SBP	-	12mm (+)	-	12mm (+)	14mm (+)	14mm (+)

Agar well- 10mm
10mm~14mm (+)
15mm~19mm (++)
20mm above (+++)
Source: Original Research

Table 5. EDXRF Analysis for Trace Elements in Thanakha.

Analysis	Shimataung (%)	Shwebo (%)	Pakokku (%)
Ca	83.185	47.510	72.521
Fe	6.872	39.660	18.336
K	4.508	2.512	3.734
Sr	4.360	1.939	5.409
Cu	1.075	1.737	-
Mn	-	0.941	-
Pb	-	-	-
Zr	-	-	-
Ti	-	3.612	-
Zn	-	2.088	-
As	-	-	-
Hg	-	-	-

Source: Original Research

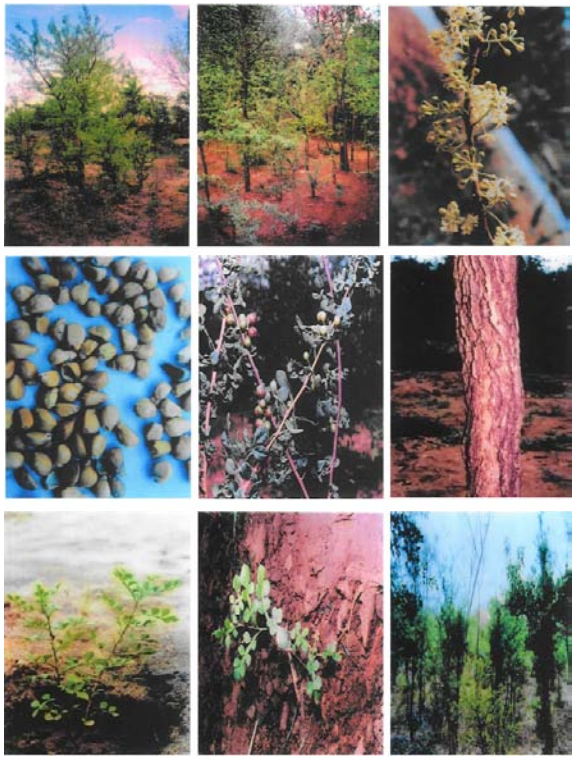

Figure 1. Shinmataung Thanakha (*Hesperethusa Crenulata* (Roxb.)M. Roem).

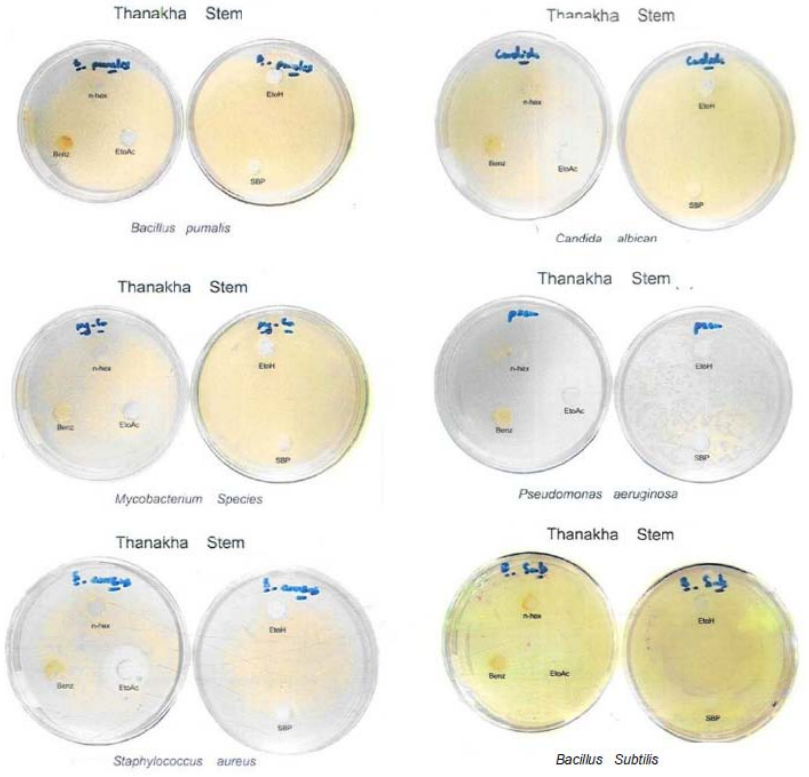

Figure 2. Anti-microbial Activity of Different Solvent Extracts from Shinmataung Thanakha (Stem).

Source: Original Research

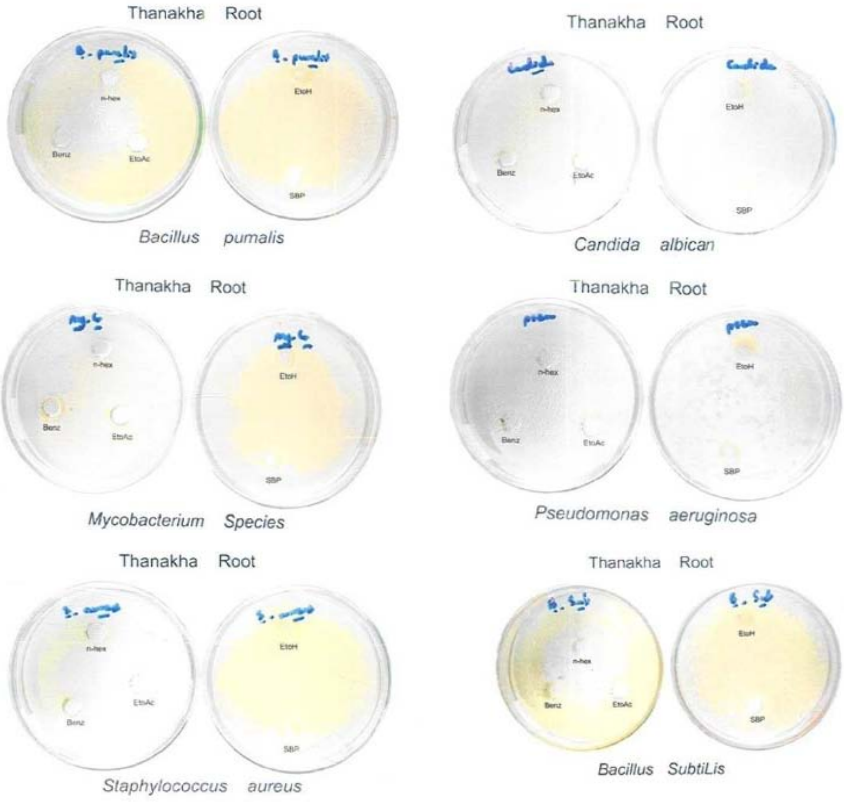

Figure 3. Anti-microbial Activity of Different Solvent Extracts from Shinmataung Thanakha (Root).

Source: Original Research

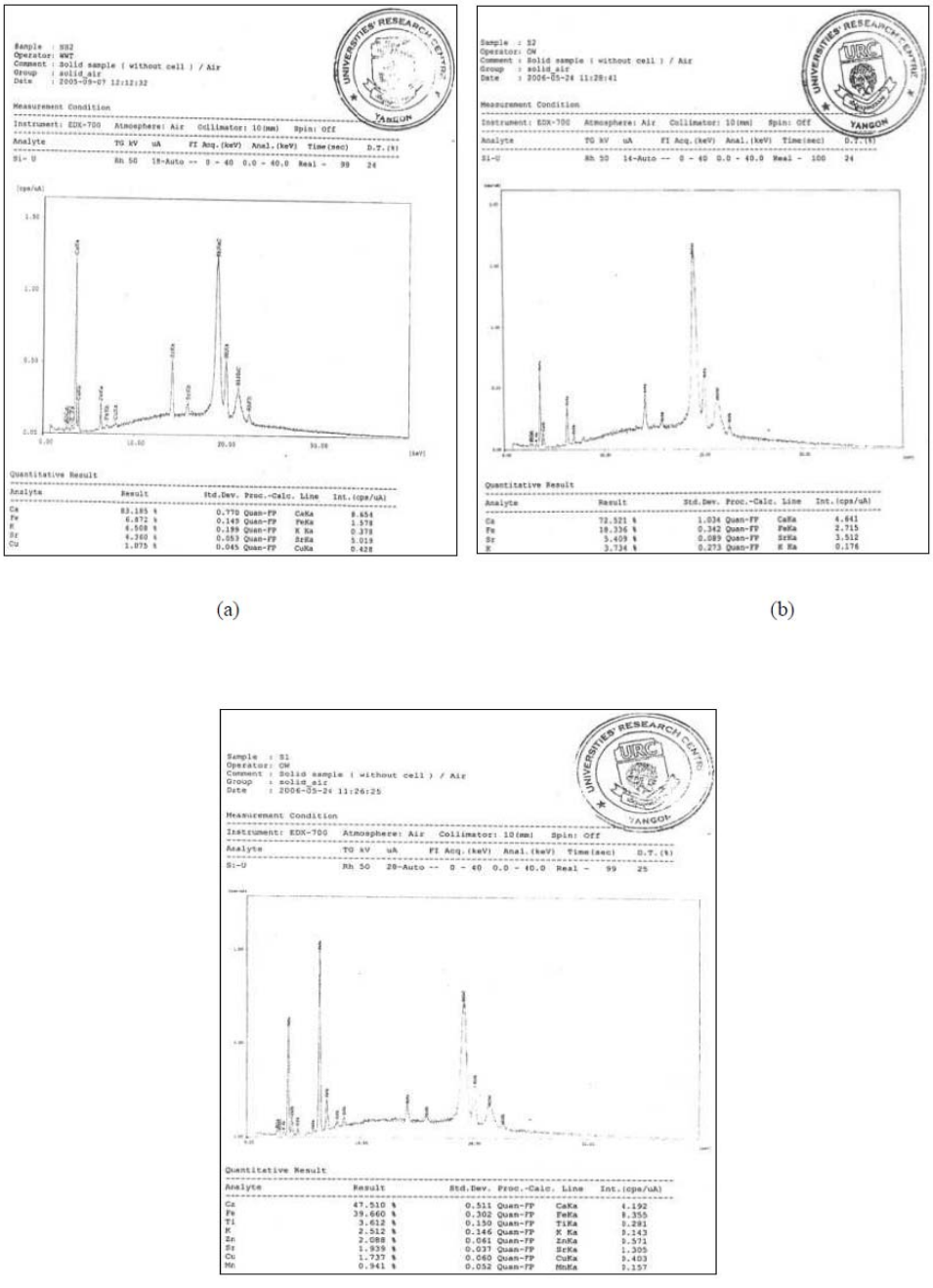

Figure 4. EDXRF Graph of Thanakha (a)Shinmataung (b) Pakokku (c) Shwebo.

Source: Original Research

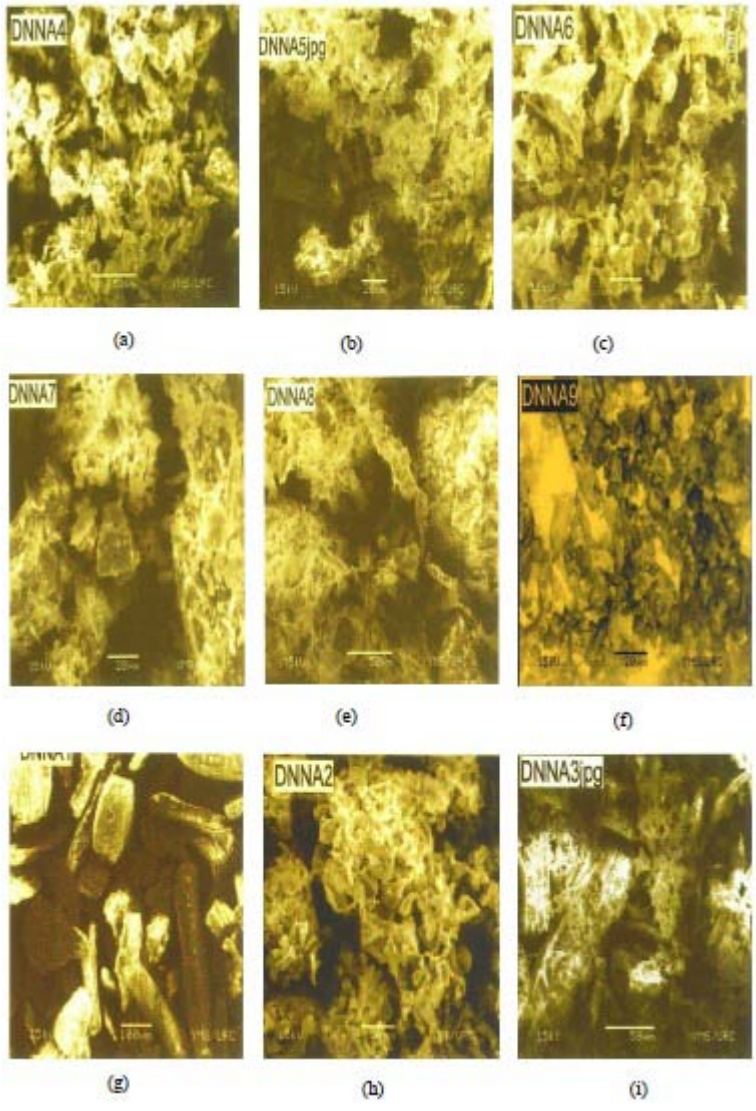

Figure 5. SEM Analysis of (a) Shinmataung (Heartwood Powder), (b) Shwebo (Stem Bark- Powder), (c) Pakokku (Stem Bark Powder), (d) Shinmataung (Stem Bark Powder), (e) Thanakha Powder from Paste (by machine) (f) Thanakha Powder from Paste (by manual,)(g) Thanakha powder (+200 mesh) ,(h) Thanakha powder (+300 mesh) and (i) Thanakha powder (-300 mesh).

Source: Original Research

CONCLUSION

Phytochemical analysis of all the solvent extracts revealed the presence of alkaloids, glycosides, saponin glycosides, steroids, phenolic group, flavonoids and tannins and absence of reducing sugars. Different solvent extracts from Shinmataung Thanakha showed anti-microbial activities against certain microorganisms. The presence of toxic heavy metals like mercury and arsenic were not found in Thanakha from different regions.

In the preparation of finished Thanakha products, it was noticed that the particle size and fibre content are of important factors concerning especially the Thanakha cream. The stability and texture of Thanakha cream depend on the particle size and fibre content of Thanakha powder. Using Thanakha heartwood only is economical but it gives impaired quality of Thanakha Products.

In conclusion, the consumers receive natural products of specified best quality by using Thanakha which has valuable properties for anyone at any age.

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