International Journal of Advanced Research and Review

www.ijarr.in

PHYSICOCHEMICAL ANALYSIS AND PHYTOCHEMICAL EVALUATION OF KOKILAKSHA (*ASTERACANTHA LONGIFOLIA* NEES)

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ABSTRACT

Asteracantha longifolia Nees family Acanthaceae is commonly known Kokilasha found in moist places, banks of rivers, ditches and paddy fields. It is also found in India, Burma, Africa, Malaysia Sri-Lanka, Myanmar, Indonesia, and Nepal. *Asteracantha longifolia* extensively used in traditional system of medicine to treat various ailments and this plant mentioned in Ayurvedic treatise like "Charka Samhita" and "Sushruta Samhita" as *Rasayan* or rejuvenator. *Asteracantha longifolia* is used for the treatment of several diseases such as jaundice, dropsy, dysentery and diabetes, rheumatism, edema, inflammation, kidney stones, pain, aphrodisiac, liver dysfunction, malaria, gout, urinary affections, dysuria, cough, diarrhoea, thirst, urinary calculi, urine discharges and treatment of blood disorders and also used to preparation of herbal formulations. On the basis of therapeutic characters a detailed study of Kokilasha different parts (root, stem and leaf) such as macroscopy, microscopy, and powder microscopy studies, physicochemical parameters, preliminary phytochemical screening, development of HPTLC fingerprints profile and detection of heavy metals, tools were carried out. The established parameters can be used as standards for identification and quality control of the plants in compound formulations and also preparation of a monograph of the plant Kokilasha plant.

Keywords: *Asteracantha longifolia*, Physico-chemical studies, Phytochemical screening, HPTLC fingerprints profile

INTRODUCTION

Asteracantha longifolia is an annual strong and sub- shruby herb that grows best in marshy areas near waterways. It is found in India, Burma, Africa, Malaysia Sri-Lanka, Myanmar, Indonesia, and Nepal. The sprout bears eight leaves and six thorns at each node on the reddish-brown stem. The leaves are sub-sessile and have minutely dentate margins with sharp, straight or curved thorns. The outer pair of leaves is bigger, oblong lanceolate, and scalerous. 1.5-4 cm long, yellowish-brown spines. Brownish-yellow flowers occur in leafy bract and bracteoles, axillary whorls. The calyx has four lobed, uneven lobes. Corolla: 5 petals, gamopetalous, unevenly 2-lipped, palate yellow in the central lobe of the lower lip; corolla purple. There are four stamens in a pair with uneven filaments, the anthers are divergent, the

ovary has two cells, with four ovules in each. Fruit dehiscent capsule linear-oblong, two-celled, compressed, pointy, and bearing four to eight seeds per cm. Ovoid, compressed, hairy, and measuring 0.2-0.25 cm in length and 0.1-0.15 cm in width, the seed is light brown in colour and tastes slightly bitter, with no overt odour when soaking in water (Hussain et.al., 2011, Chopra et. al., 2006, Sharma et.al., 2002 and Saxena & Brahmam 2002). *Asteracantha longifolia* extensively used in traditional system of medicine to treat various ailments and this plant mentioned in Ayurvedic treatise like "Charka Samhita" and "Sushruta Samhita" as Rasayan or rejuvenator. It is classified in ayurvedic system of medicine as Mathuravipaka and Seethaveeryam. Whole plant is used for the treatment of several diseases reported by different researchers such as jaundice (Agarwal, et.al., 2014) dropsy, (Hutchings, et.al., 1996) dysentery and diabetes (Kuru, P. 2014) rheumatism (Hussain et.al., 2011) edema (Govindachari et.al., 1957), inflammation (Hewawasam, et.al., 2003), kidney stones (Arjun, et.al., 2010), pain (Pattanayak & Sunita 2008), aphrodisiac, liver dysfunction (Ahmed et.al., 2001), malaria (Mazumdar et. al., 1997), impotence (Buvaneswari,et.al., 2011), hepatic obstructions (Pareek & Barthakur, 2014), dissolution of gallstones (Patra et.al., 2009), gout (Parashar & Singh, 1965), diseases of urinogenital tracts (Balasubramanian & Premkumari, 2012), plant ash used against gravel and dropsy (Boily & Vnpuyvelde, 1986), whole plant is beneficial in urinary affections, dysuria, and painful micturition (Valientic et.al., 1995), root decoction used to treat gonorrhea and rheumatism (Patra et.al., 2008), the leaves have diuretic, sweet, tonic, aphrodisiac, and hypnotic properties. They can be used to treat inflammations, cough, diarrhoea, thirst, urinary calculi, urine discharges and spermatorrhoea (Singh & Handa 1995). The seeds are used as ingredients in various preparations and in treatment of blood disorders, biliousness, gonorrhoea, spermatorrhea and fever (Kumar et.al; 1999). The plant contains a diversity of biologically compounds such as alkaloids, flavonoids, terpenoids, vitamins, carbohydrates, glycosides, waxy substances, gum, phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, enzymes, amino acids, hydrocarbons, reducing sugars, essential Oils, gums etc. minerals like Calcium, Potassium, Iron, Zinc, Manganese, Magnesium, Chromium, Copper and Cobalt present in various part of this plant. Keeping this aim into consideration, the present study was designed to scientific evaluation of *Asteracantha longifolia* different parts. The study includes macro and microscopic characters, powder microscopic characteristics, High Performance Thin Layer Chromatography fingerprints (HPTLC), preliminary phytochemical screening and physicochemical parameters. The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of Kokilaksha.

MATERIAL AND METHODS

Plant materials collection and preparation of sample

Asteracantha longifolia Nees. different parts were collected from Satianusuiya Ashram, Chitrakoot, Satna (M.P.) in the month of December, 2021.Samples were identified and authenticated by Dr. Manoj Kumar Tripathi SRO & Head,(R&D), Arogyadham, Deendayal Research Institute Chitrakoot. The voucher specimen prepared (specimen no. Govt./PGC/549) as per standard procedure (Jain SK & Rao, 1977) and maintained in the unit of herbarium under department of botany, Government Autonomous Post Graduate, College, Satna (M.P.) for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical

investigation and development of High Performance Thin Layer Chromatography fingerprint profile.

Macroscopic and Microscopic study

Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated. Fresh root bark section was cut by free hand sectioning and numerous sections examined microscopically (Sholapur and Patil et al. 2013). Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 21*l* with Digi-eye camera using Caliper plus version 4.2 software.

Powder microscopic study

The dried root bark was subjected to powdered and completely passes through 355 µm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 µm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerine, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin. Treat a few mg with iodine solution and mount in glycerine, about 1 g of powder warmed over water bath with Chloral hydrate solution till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40X x 10X magnification of the Trinocular Research Microscope (Anonymous, 2000).

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, hexane soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were calculated (Tripathi & Sikarwar, 2015).

Heavy metals tests

Heavy metals are toxic and generally occur through earth in plants. Mainly four types of heavy metals harmful for us they are Pb, Cd, As and Hg. These heavy metals detected through Atomic Absorption Spectrophotometer as per standard method (Ansari S H, 2006).

Preliminary phytochemical studies

Preliminary phytochemical tests were carried out on various extracts for the presence\absence of phyto-constituents like alkaloids, flavanoids, steroids, terpenoids, tannins, resins, carbohydrates, proteins and saponins (Choudhary et. al. 2014).

High Performance Thin Layer Chromatography (HPTLC)

For High performance thin layer chromatography, the powdered 5 gm of each samples (root, stem and leaf) were extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 8 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of *Toluene: Ethyl acetate (7:3* v\v). Linear ascending development was carried out in 20x10cm twin through glass chamber

equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, Thin Layer Chromatography plate was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at 254nm, 366nm and day light with Win cat software and R_f values noted (Tripathi et.al. 2015).

RESULT AND DISCUSSION

Macroscopic and Microscopic characters

Asteracantha longifolia (Kokilaksa) family Acanthaceae is a spiny, stout, annual herb, commonly grow in water logged places found throughout the country. Leaves are subsessile, oblong-lanceloate or linear lanceolate, spines yellowish brown, 2-3 cm long, flower yellowish brown, fruit two celled, linear oblong, compressed about 8 cm long, pointed, 4-8 seeded. Seed ovate, flat or compressed, 0.2-0.25 cm long and 0.1-0.15 cm wide, hairy but appearing smooth; when soaked in water immediately get coated with mucilage, light brown, taste slightly bitter and odour not distinct. Kokilaksa root colour is whitish to brownish, odor characteristics and taste slightly bitter. Root mostly adventitious and branched. Stem usually unbranched, fasciculate, sub-quadrangular swollen at nodes covered with long hairs which are numerous at the nodes. Externally grayish brown color, creamish brown in cut surfaces. Odor and taste not characteristics and eaves color is greenish brown, 1.5-9 cm long, 0.5-1.5 cm wide, subsessile, lanceolate, acute, entire and hairy, (Fig. 1-4).

Fig.1Plant

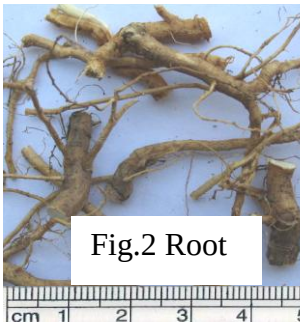

Fig.2 Root

Fig.3 Stem

Fig.4 Leaf

calcium oxalate, oil globules, parenchyma filled with brown contents, cork cells in sectional view, cork cells in surface view filled with brown contents, cork cells in sectional view, Parenchyma filled with brown contents, simple pitted vessels, tracheids with simple pits, fibres and parenchyma filled with starch grains. Stem powder is greenish brown color with pleasant odor and slightly pungent taste. Under microscope powder shows pitted vessels, reticulate thickenings, scalariform thickening, spiral thickening, prismatic crystals of calcium oxalate, trichomes, parenchymatous cells filled with starch grains, starch grains, epidermis in surface view, aseptate fibres and fragments of medullary rays crossing the fibres. Leaf powder color is green taste and odor not characteristics, under microscope shows various shape size of trichomes, spongy parenchyma embedded with oil globules, pitted vessels, acicular crystals of calcium-oxalate, starch grains, collenchymatous cells, upper epidermis with stomata, lower epidermis with stomata, palisade cells and epidermal cells.

Physico-chemical analysis

The physico-chemical tests such as Loss on drying on 105^0C, solubility such as water soluble extractive values, alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. The results are expressed as mean (n=3) ± standard deviation in w/w. The loss on drying value obtained is an indicative of amount of moisture content could prevent bacteria, fungal or yeast growth.Water soluble extractive value is higher than the alcohol soluble extractive value. The extractive values, indicates the amount of active constituents in given amount of plant material when extracted with respective solvent and useful for the determination of exhausted or adulterated drug. Ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of the ingredients. Ash value is useful in determining authenticity and purity of the drug and also these values are important quantitative standards. The results of physicochemical analysis are given in (Table 1). It is observed that the leaf LOD value 6.45% is higher than the stem LOD 5.42% and root LOD 4.95%. Respectively total ash values were higher in root (8.26%) as compared to leaf (7.80%) and stem (3.04%). While, root acid insoluble ash is higher 6.97% than leaf 5.72% and stem 1.43%. Water soluble extractive value were higher than alcohol soluble extractives in leaf (17.50, 7.50%W/W), stem (15.47, 11.27%W/W) and root (15.56, 11.55%W/W).

Table -1: Physico-chemical analysis of *Asteracantha longifolia*

Name of ingredients	**LOD (% w/w)**	**Mean (%)**	**Total ash (% w/w)**	**Mean (%)**	**AI ash (% w/w)**	**Mean (%)**	**ASE (% w/w)**	**Mean (%)**	**WSE (% w/w)**	**Mean (%)**
Root	5.14 4.83 4.88	**4.95**	8.26	8.26	6.97	6.97	10.20 11.50 11.95	**11.55**	15.30 15.38 16.00	**15.56**
Stem	5.32 5.30 5.65	**5.42**	3.04	3.04	1.43	1.43	11.25 11.08 11.58	**11.27**	15.501 15.68 15.25	**15.47**
Leaf	6.30 6.74 6.33	**6.45**	7.8	7.8	5.72	5.72	7.55 7.35 7.6	**7.5**	18.53 17.66 16.31	**17.50**

Preliminary phyto-chemical investigation

Preliminary phyto-chemical analysis was performed in petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water of *Asteracantha longifolia* root, stem and leaf powder was carried out. Alkaloids, resins, flavonoids, glycosides, resins, carbohydrate, are present in the root, in stem present terpenoids . Alkaloids, resins, flavonoids, glycosides while flavonoid, tannin, glycoside, proteins are present in the leaf.

HPTLC finger print profile

Samples were applied by spotting test solutions 8 µl on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5

sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part, and apply 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 µl standard marker Quercetin and Stigmasterol 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe and standard markers, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin the plate and 10 mm part. The plate was developed using a mobile phase consisting of *toluene: ethyl acetate* (7:3v/v). Linear ascending development was carried out in a 10x20cm twin through glass chamber equilibrated with the mobile phase. The optimized chamber saturation time for the mobile phase (20 ml) was 30 min at room temperature. The length of the chromatogram run was 85 cm. Subsequent to the development, a thin layer of chromatography plate was dried at room temperature. The peak area for samples and standards were recorded with the camera photo documentation system Camag Reprostar 3 and the plate was scanned densitometrically with the help of Scanner 4. Record the respective areas and prepare a calibration curve by plotting peak area *vs* concentration of standard markers Quercetin and Stigmasterol. It is observed that the Quercetin is higher present in *Asteracantha longifolia* root than the stem and leaf, while Stigmasrerol was higher present in *Asteracantha longifolia* stem than the root, but absent in the leaf.

The percentage of Quercetin ranges from 0.025 to 0.32 percent in the Quercetin root analyzed, while Quercetin range was found in *Astercantha longifolia* stem and leaf, 0.021 to 0.024 percent and 0.018 to 0.021 respectively. And the percentage of Stigmasterol ranges from 0.009 to 0.12 percent in the *Asteracantha longifolia* stem analyzed, while 0.05 to 0.09 percent in root, but absent in leaf (Fig.5, 6 &7).

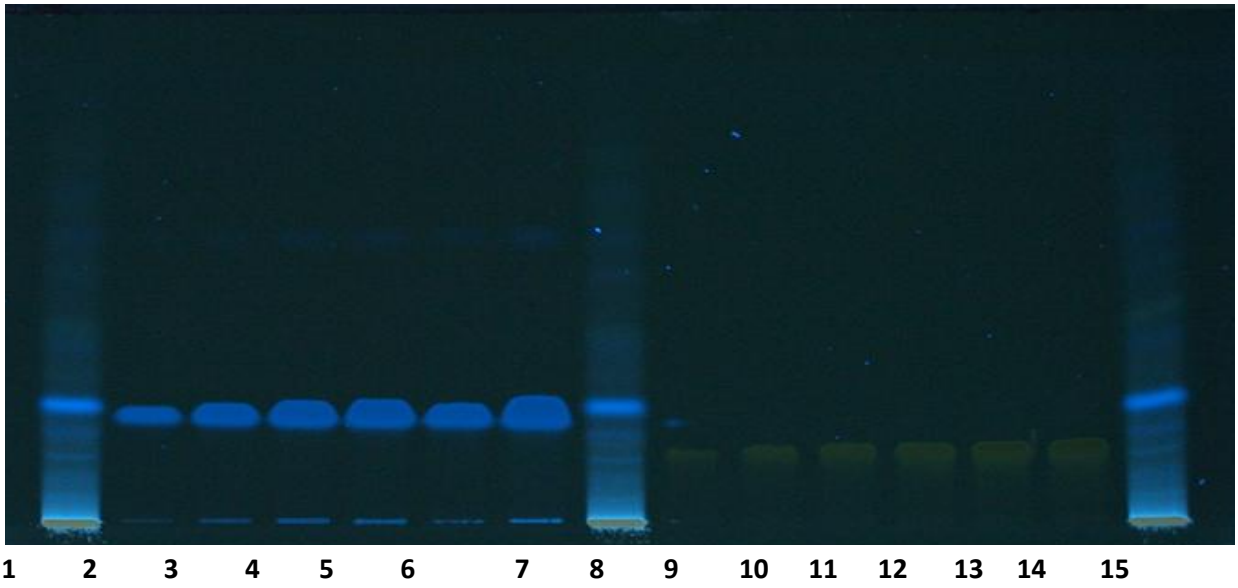

Fig.5 HPTLC fingerprints profile of Asteracantha longifolia at366nm.
Abbreviation- Track 1: test solution of Asteracantha longifolia root; Track 2-7 Quercetin standard; Track 8: test solution of Asteracantha longifolia stem; Track 9-14: Stigmasterol standard; Track 15: test solution of Asteracantha longifolia leaf.

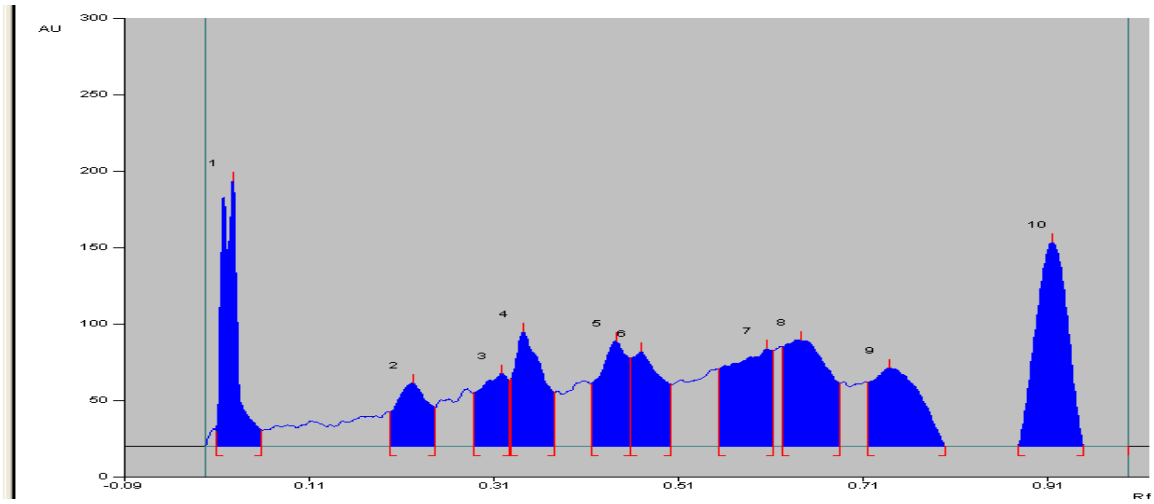

Fig.6: Standard Peak of HPTLC Fingerprint profile of test solution of *Asteracantha longifolia*

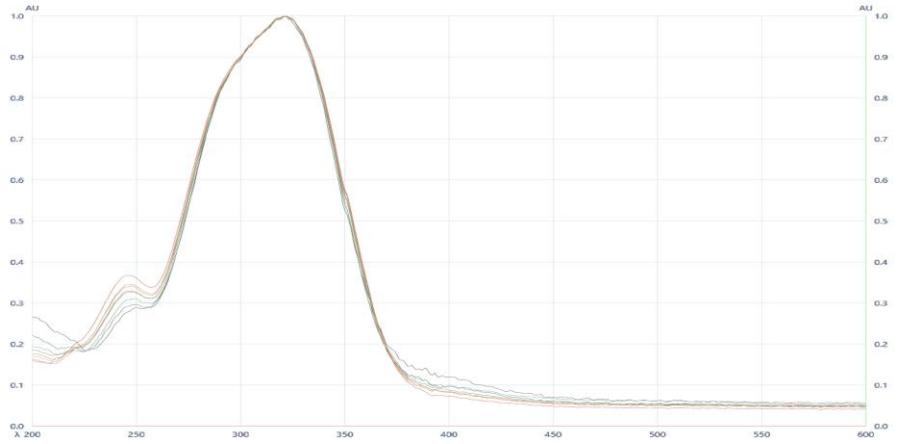

Fig.7: Calibration curve of HPTLC Fingerprint profile of test solution *Asteracantha longifolia*

Heavy metals tests

Heavy metal elements (Pb, Cd, As and Hg) test were performed and found under limits as per guideline WHO.

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify *Asteracantha longifolia* root, stem and leaf. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate's important phyto-constituents. Heavy metal elements are found under limits as per guideline WHO. All findings are indicating samples are genuine

and free from any adulterations. These finding could be helpful in identification and authentication of *Asteracantha longifolia* root, stem and leaf

CONCLUSION

Various references showed the importance of *Asteracantha longifolia.* Various parts of *Asteracantha longifolia* plant are used to treat different types of ailments like jaundice, dropsy, dysentery and diabetes, rheumatism, edema, inflammation, kidney stones, pain, aphrodisiac, liver dysfunction, malaria, gout, urinary affections, dysuria, cough, diarrhoea, thirst, urinary calculi, urine discharges and treatment of blood disorders and also used to preparation of herbal formulations. Hence, there would be no exaggeration in concluding that this plant is accepted as most sacred for its high medicinal values.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Shivesh Pratap Singh, Principle, Government Autonomous. P. G. College, Satna, Madhya Pradesh, India and Shri Abhay Mahajan, Organizing Secretary, Deendayal Research Institute, Chitrakoot, Sarna (M.P.) for providing infrastructure and necessary facilities during research tenure.

Conflict of interest: Authors declare no conflict of interest.

Statement of ethical approval: No issue.

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