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Quantitative assessment and antibacterial activity of *Origanum vulgare* L.

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Abstract
Biodiversity, which twenty years ago was considered unimportant by most ecologists, has now been shown to impact significantly upon many aspects of ecosystem functioning. Diversity must now be added to the list of factors — including species composition, disturbance regime, soil type and climate — that influence ecosystem functioning. The recent knowledge of the importance of biodiversity highlights an under-appreciated truth — although society is dependent on natural and managed ecosystems for goods and services that are essential for human survival, we know all too little about how ecosystems work. *Origanum vulgare* L. is one of the important ethno medicinal plants, are found spread over to sub-temperate forest zone. They are of Ayurvedic importance and have trade value as well. Due to over exploitation and habitat degradation they are now turning into a rare species. Threat assessments of this species revealed that they are vulnerable in study area under reference. Their dominance too was found less as compare to other species. Their diversity profile in study areas also went down. *Origanum vulgare* L. has antimicrobial activities but *Becillus subtillis, Pseudomonas aereuguinosa, Staphylococcus aureus* and *Escherchia coli bacteria were shown to be proactive.* Due to fast growing urbanization of life, people are being attracted towards use of natural products and as such utility of such plants are gaining ground. Thus the need is to protect, promote and conserve such natural resources to have advantage of biodiversity conservation.

Keywords: Antibacterial activity, Ethno-medicine, Population assessment, *Origanum vulgare* L.

INTRODUCTION

The existence of a great diversity of species on earth remains a mystery till date. Its solutions too are inviting and attaining new heights every day. To explain why and how biodiversity influences the functioning of ecosystems, therefore, is always challenging. One of the answers may lie in quantifying the trade-offs that organisms face in dealing with the constraints of their environment. Societal responses to the loss of biodiversity also involve trade-offs, and the elaboration of these are essential in developing wiser environmental ethics and policy. Therefore the most striking feature of earth is the existence of life, and the most striking feature of life is its diversity. This biological diversity, or biodiversity, has long been a source of wonderment and scientific curiosity, but is increasingly a source of concern. Human domination of earth's ecosystems is markedly reducing the diversity of species within many habitats worldwide, and is accelerating extinction[1]. One of the more pragmatic questions raised by these threats to biodiversity is the extent to which this loss of biodiversity matters; that is, are stability, productivity and other aspects of the functioning of both managed and natural ecosystems dependent on biodiversity?

Origanum vulgare L.[2,3] is a perennial, aromatic, hairy herbs; 25-90 cm high belonging with Lamiaceae family. Local name of this plant is 'Bantulsi'. Habitat of *Origanum vulgare* L. is open forest localities and Waste places (Figure 1: *Origanum vulgare* L). The nativity of *Origanum vulgare* L. is Europe As et Afr Trop. Oregano plays a primary role among temperate culinary herbs in world trade [4]. It is native of Southern Europe. It is cultivated in European countries and is one of the most popular herbs in Mediterranean cooking. It is traded both as 'whole' dried leaves and in ground form [5]. The leaves and dried herb of oregano as well as its essential oil are used medicinally [6]. The volatile oil of oregano has been used traditionally for respiratory disorders, indigestion, dental caries, rheumatoid arthritis and urinary tract disorders [7].

In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Antimicrobial resistance is a threat to mankind because most of the infection causing bacteria has become multidrug resistant. Antibiotic resistant bacteria may keep people sick longer, and sometimes people are unable to recover at all. Because of the concern about the side effects of conventional medicine, the use of natural products as an alternate to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. The knowledge and expertise in folk remedies conserved by remote hilly area peoples need to be documented and investigated for modern drug therapeutics [8-10]. Due to lack of modern medical facilities, expensive drugs and poor transportation, patients of these localities normally suffers for a long. In these unfavorable situations traditional herbal healers of this remote locality play a vital role to provide them as an alternate source of therapeutic facilities for their primary healthcare. Subsequently, with the advanced in the techniques of phyto-chemistry and pharmacology, a number of active principles of medicinal plants were isolated and introduce as valuable drugs in modern system of

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medicine. The manuscript describes distribution and local/traditional uses of the medicinal flora and identifies the plants that need conservation and protection.

It can't be denied that in ancient day when medical facilities were not so developed used to depend on traditional knowledge system [8-11].The dormant knowledge during the ancient time definitely was solid and was helpful in curing different diseases but that is now not in vogue in. It now needs revival research and further development to make herbal medicine knowledge savvy. It also needs conservation of traditional knowledge system so that a fund of disciplined knowledge savvy system be created and a continuum of furtherance of preservation and strengthen this knowledge system. The structure of community can be studied by taking into consideration a number of characters which are usually grouped. Certain analytical characters like frequency, density, abundance, concentration of dominance, species richness and species diversity can be expressed quantitatively. The bio-efficacy of *Origanum vulgare* L. has been examined against the growth of bacteria in vitro to evaluate their anti-microbial potential.

Fig1.*Origanum vulgare* L.

METHODS

Study area

Ranikhet is a hill station and cantonment town in the Indian state of Uttarakhand. Ranikhet is situated at the latitude $29^0$30' to 29^0 40' N and longitude 79^0 19' to 79^0 35 E in a central Himalayan region (Figure 2: Google map of Ranikhet, India). The entire area is hilly with altitude ranging from 900-2115 m. Average rainfall recorded is 1300 mm and over 75% rainfall occurs during monsoon period (July to September). October and November are usually dry months followed by winter rain. January- February are the coldest months experiencing snow fall. The mean maximum temperature is 32^0C in May-June and lowest 3^0C in January. Based on climate, topography and altitude, the vegetation met with in different parts of the area, ranges from sub mountain to mountain type.

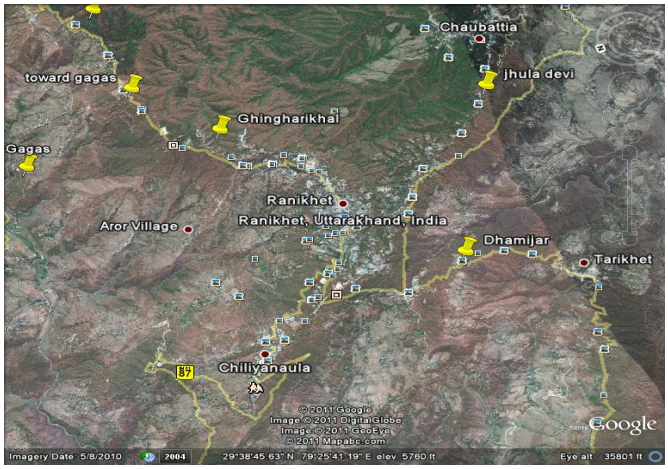

Fig 2. Study sites of Ranikhet, India

Methodology of quantitative assessment

The main purpose of this survey was to document the traditional knowledge. Information about the medicinal use of plants was collected through surveys conducted in several regions of Ranikhet, India. During the field surveys, attempts were made to cover all major habitat types available in the study area. These people are apparently very friendly but maintained a deep secrecy about their traditional knowledge. However, after visiting them for several times, they finally shared their knowledge about the medicinal uses of *Origanum vulgare* L. growing around them for disease. After that the plants were identify at Regional Research Institute of Himalayan Flora, Tarikhet Uttarakahnd (India). To know the population of these plants field investigation has been done. During field survey, study area was divided into three zones on the basis of altitudinal range i.e. Area-1(900-1300m), Area-2(1300-

1700m) and Area-3(1700-2115m). Vegetation analysis of the selected areas along an altitudinal gradient was carried out by using 1×1 m quadrates randomly. The size and the number of quadrates were determined [12]. At different aspects 30 random quadrates each of 1×1 m for herbs were laid out for phyto-sociological study. The vegetational data were quantitatively analyzed for abundance (A), density (D), frequency (F) [12]. In this study PAST software is used to analyses the diversity profile and cluster analysis of study area to interpreting the results. The main aim of this study is to identify the status and nativity of ethno medicinally important *Origanum vulgare* L. of Ranikhet, India

Plant material for anti bacterial studies
Preparation of Infusion

The infusion was prepared by taking 10 g dried leaves of oregano in 100 ml absolute alcohol and left for 24 h at room temperature with constant stirring and filtered to obtain clear infusion.

Essential Oil Extraction

The leaves of oregano (40 g) were dried in shadow at room temperature and distilled for 2h. The yellowish oil (1ml) of leaves was dissolved in n-hexane and then dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at 40°C and the pure oil was kept in refrigerator until the moment of analysis.

Antibacterial tests

In vitro antibacterial activity of the infusion (test compound) against *Becillus subtillis, Pseudomonas aereuginosa, Staphylococcus aureus and Escherchia coli* were carried out using paper disc diffusion method. The nutrient agar medium (peptone, beef extract, NaCl and agar-agar) and 5mm diameter paper discs of Whatman No.1 were used. The test compound was dissolved in ethanol in 0.1-0.4% concentrations. The paper discs were soaked in different solutions of the compound, dried and the placed in the Petri plates (9 mm diameter) previously seeded with the test organisms, *Becillus subtillis, Pseudomonas aereuginosa, Staphylococcus aureus and Escherchia coli.* The plates were incubated for 24 – 30 h at 27 ±1 °C and the inhibition zones (mm) were measured around each disc. As the organism grows, it forms a turbid layer, except in the region where the concentration of antibacterial agent is above the minimum inhibitory concentration, and a zone of inhibition is seen. The size of the inhibition zone depends upon the culture medium, incubation conditions, rate of diffusion and the concentration of the antibacterial agent. The minimal inhibition concentration (MIC) values were evaluated with micro-organisms that displayed inhibitory zones. MIC was determined by dilution of the essential oils in DMSO within the concentration range 0.10-2.00 mg/ml.

RESULTS AND DISCUSSION

In the study area, type of forest *Bouhinia retusa* Ham ex. Roxb is dominant and other important floras are *Eugenia dalbergioides* Benth., *Anogeissus latifolia* (Roxb. ex DC) Wall. ex.Guill. & Perr., *Mallotus Philippensis* (Lam.) Muell.-Arg.,*Sapium insigne* Benth.,*Gochnatia spectabilis* (D. Don) Less.,*Engethardtia spicata* Leschen. Ex. Bl..,*Erythrina suberosa* Roxb.,*Pistacia chinensis* Subsp, *Nyclanthes arbertristis* Linn. and *Oleaglandulifera* Wall. ex G. Don. In temperate forest, the pine is the dominant species, but in northern region where there is more moisture in the soil, various species occur. The ground is always covered with a more or less dense crop of grasses during rains. Other associates of this forest is *Quercus leucotrichophora, Rhododendron arboretum, lyonia oralifolia, Myrica esculenia, Pyrus pashia, Pyracantha crenulata, Aluns nepalensis, Symplocos chinesis, Syzgium cumini, Phus parviflora, Engelhardtia colebrookeana, Ougeinia Oojeinensis, Emblica officinalis, Ficus auriculata, Glochidion velutinum* are among the tree species. *Euphorbia royleana* is very often abundant on rocky southern and *Rhus parviflora* and *Woodfordia fruticosa* sometime from on undergrowth of varying density and extent in the area subjected to heavy grazing and looping. The most common shrubs in this forest are as follows: *Indigofera dosua, Indigofera cassioides, Flemingia fruticosa, Lespoedeza stenocarpa, Rubus ellipticus, R. niveus, Achymenthera tomentosa, Myrsine Africana, Indula cappa* etc. Ranikhet is vegetationally rich place, out of all vegetations there is so many plants with ethno medicinal values. The previous work reports the distribution and status of 60 species (56 genera belonging to 38 families) of ethno medicinal plants [13]. Ethno-medicine has emerged as an important branch of study which focuses on the utility of different plant species and their properties as food, medicine and other uses [14,15]. Over the past few decades, the traditional knowledge on the use of medicinal plants has been widely acknowledged across the world. According to the World Health Organization, 80% of the world's population is developing country using traditional medicines [15].

Ethno-medicinal notes

It has a beneficial effect upon the digestive and respiratory systems. It is also used to promote menstruation. Mixture of plant extract with Ginger extract in equal amount and this mixture are heated. This is taken orally with honey and is very useful in bronchitis, colic and diarrhea. Leaves are eaten as vegetable and flavouring agent. Flowering branches hung on the houses to get rid of bad spirits. Decoction of whole plant is given orally in urinary disorders. Paste of whole plant is mixed with turmeric paste/ powder in 3:1 in ratio and applied externally in skin disease. Paste of leaves with honey is useful in pimples. Seed of plants is heated in mustard oil and massage with it, very useful in body pain and cramps. It is restricted in high dose and also restricted in pregnancy. All areal part is very useful except the root. The fresh parts are more effective and efficient for use.

Quantitative Assessment

The loss, decline or fragmentation of habitat through excessive clearing of native vegetation poses a significant threat to flora and funa [16,17]. Many such species are at the verge of extinction due to evolutionary trends or man made threats, of which latter is predominant factor during past few decades. H. intermedia is known as 'Riddhi' in ayurveda is identified as endangered plant in Western Himalaya[18]. On the basis of observation, status of *Origanum vulgare* L. is rare in Ranikhet, India. Phytosociological data reveals that *Origanum vulgare* L. having highest frequency (73.33) in area-3. Relative frequency of area-1, 2 and 3 are 3.94, 4.23 and 5.34 respectively, while the area-3 having highest density than other areas. Relative density, abundance and species richness

of area-3 is also higher than area-2 and area-3. Concentration of dominance is almost similar in all the areas. Species diversity of area-1 and area-3 is similar and higher than the area-2. Performance detail is presented diagrammatically in Figure 3. Distributed of *Origanum vulgare* L. is random in all the areas based on the A/F ratio. The value of IVI (Figure 4) is representing the dominance of species in an area. The range of IVI of *Origanum vulgare* L. in Ranikhet(India) is 5.53–11.40. This range of IVI is very less in all the areas which reflect the less dominance of this species in all area of Ranikhet, India. These phytosociological data reveals the decreasing diversity profile (Figure 5) of *Origanum vulgare* L. in Ranikhet, India

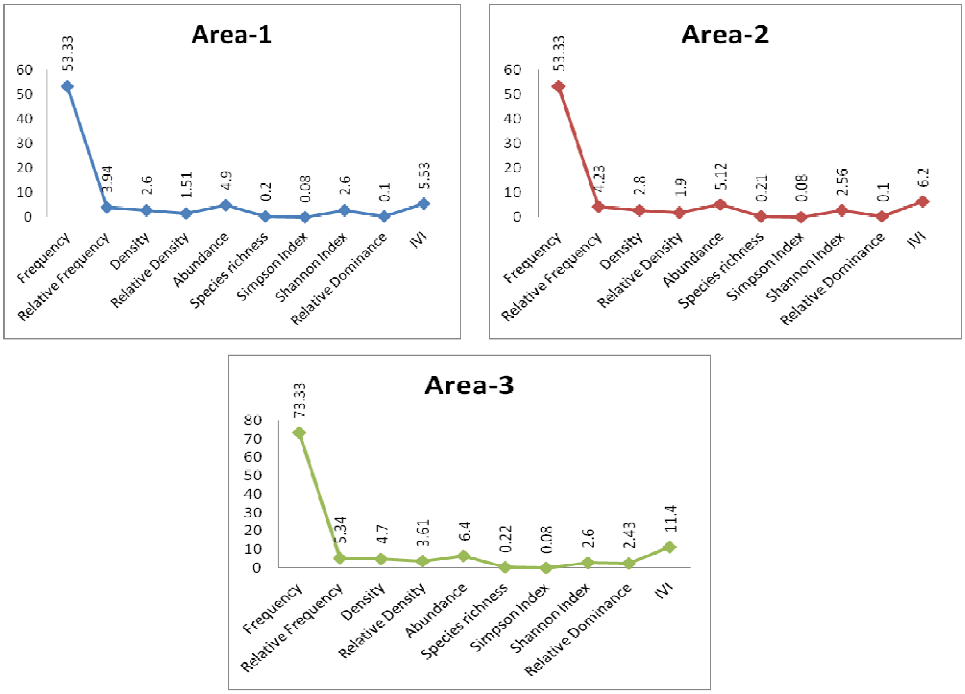

Figure 3: Area-wise performance detail

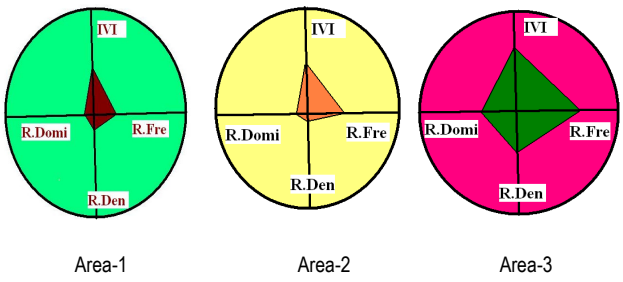

Fig 4.Phytograph of *Origanum vulgare* L. in different areas

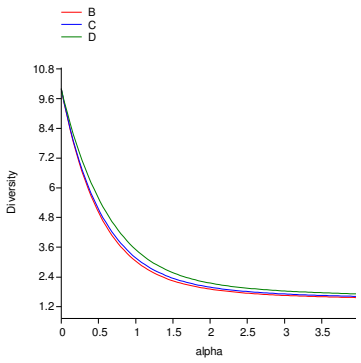

Fig 5. Diversity profile of *Origanum vulgare* L.

Threat Assessment

A large number of studies have been carried out on medicinal plants of the IHR[8-11,19-26]. The threat category of a species was identified using six attributes (i.e. habitat preference, distribution range, population size, use pattern, extraction trend, native and endemic species) [22-29]. Species with a combination of these criteria (serial number 1, 2 and 3) were given marks accordingly. Species with scores 60 were identified as critically endangered; 56–60 as endangered; 51–55 as vulnerable; 46–50 as near threatened; and 46 as of least concern. Using this six attributes, *Origanum vulgare* L. score 54 in each study area of Ranikhet, India (Table 1) indicates that *Origanum vulgare* L. having status as vulnerable in Ranikhet, India

Table 1. Threat assessment of *Origanum vulgare* L.

Area	Plant Species	Habitat	Distribution	Population (Ind/Locationn)	Use Pattern	Extraction	Native and Endemic	Score
Area-1	*Origanum vulgare* L.	6 (open forest localities and Waste places)	10 (900-1300m)	10 (78)	10 (More than4)	Commercial Self Use (10+6)	2 (Non Native)	54
Area-2		6 (open forest localities and Waste places)	10 (1300-1700m)	10 (82)	10 (More than4)	Commercial Self Use (10+6)	2 (Non Native)	54
Area-3		6 (open forest localities and Waste places)	10 (1700-2115m)	10 (141)	10 (More than4)	Commercial Self Use (10+6)	2(Non Native)	54

Table 2. Site-wise performance details of *Origanum vulgare* L.

	Site	Frequency	Relative Frequency	Density	Relative Density	Abundance	A/F	Simpson's Index	Relative Dominance	IVI
Area-1	Site-1	40	2.98	1.8	1.03	4.50	0.11	0.075	0.05	4.07
	Site-2	60	4.41	2.8	1.60	4.66	0.07	0.075	0.08	6.1
	Site-3	60	4.41	3.2	1.90	5.33	0.08	0.074	0.1	6.42
Area-2	Site-4	60	4.72	3.5	2.39	5.83	0.09	0.079	0.09	7.2
	Site-5	40	3.25	2.0	1.40	5.00	0.12	0.082	0.05	4.71
	Site6	60	4.68	2.7	1.84	4.50	0.07	0.08	0.06	6.59
Area-3	Site-7	80	5.79	4.3	3.47	5.37	0.06	0.081	2.53	11.8
	Site-8	80	5.79	5.8	4.38	7.25	0.09	0.079	2.93	13.11
	Site-9	60	4.41	4.0	2.97	6.66	0.11	0.079	1.89	9.28

Clustering

The dendrogram of floristic dissimilarity produced by grouping analysis based on the different parameters / 9 variables (Table 2) used in study of species in the 9 sites of 3 areas and three clusters can be seen in Figure 6. The results demonstrate a considerable fit of the similarity and dissimilarity matrix to the estimated clustering that produce the dendrogram and the Cophenetic Correlation is 0.883. For the entire process, complete linkage (Furthest Neighbor) of clustering method, Distance Type-Euclidean and Scale Type-Range were selected. Total 8 links were identified. The nearest neighbor of dendrogram is 8th number cluster and the furthest neighbor of dendrogram is 1st number cluster which has highest linked areas. The distance value of nearest neighbor is 1.5 and highest neighbor is 27.50.

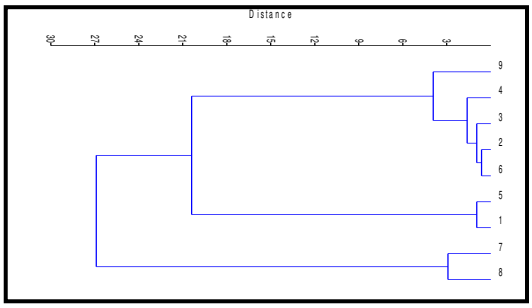

Figure 6. Cluster analysis of *Origanum vulgare* L.

Antibacterial activity

The biological activity of the oregano and chloroamphenicol (as a standard compound) were tested against bacteria because bacteriums can achieve resistance to antibiotics through biochemical and morphological modifications. The organisms used in the present investigations included Becillus subtillis, Staphylococcus aureus (as gram positive bacteria) and Pseudomonas aereuguinosa and Escherchia coli (as gram negative bacteria). The diffusion agar technique is used to evaluate the antibacterial activity of the *Origanum vulgare* L. The Oregano has moderate activity in comparison with gram positive bacteria (*Becillus subtillis, Staphylococcus aureus*) and less active in comparison with as gram negative bacteria (*Pseudomonas aereuguinosa and Escherchia coli).* The activity of the oregano increases as the concentration (0.1-0.4%) increases because it is a well known fact that concentration plays a vital role in increasing the degree of inhibition [30]. The essential oil extracted from leaves of *Origanum vulgare* L. was used in the present study to investigate their antibacterial potential. With the agar disc diffusion assay, oils were found to be active against gram positive bacteria i.e, in the range 0.12-0.16 mg/ml against gram negative bacteria whose range are 1.75-2.00 mg/mL. The MIC has the lowest concentration that inhibited the visible bacterial growth (Figure 7). On the basis of above results, it can be concluded that *Origanum vulgare* L. represents a source mixtures of antibacterial constituents that can be as effective modern medicine to combat pathogenic micro-organism.

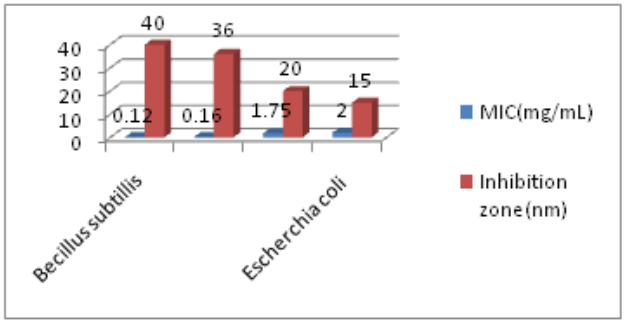

Figure 7. Antibacterial studies of *Origanum vulgare* L.

CONCLUSION

According to an estimate of the World Health Organization, approximately 80% of the people in developing countries depend on traditional medicines for primary health care needs; a major portion of these involves the use of medicinal plants. Traditional Himalayan Medicine System (THMS) is a great example of TKS where small communities prevent his life for incurable diseases through the traditional methods, which came from their fathers or grand fathers and goes to next generation. They are also curing their animals through these traditional methods also. These traditional methods are totally oral and non-documented. This is one of the most important regions of degradation of traditional knowledge system. *Origanum vulgare* L. is an important ethno-medicinal plant. This plant is also in trouble from over harvesting and destruction of habitat. Population growth, urbanization and the unrestricted collection of it from the wild is resulting in an over-exploitation of natural resources. Therefore, the management of these ethno-medicinal plant resources has become a matter of urgency. An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is potent and has opened extensive areas of research for biodiversity conservation. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species.

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