# Allelopathic Potential of Some Essential Oil Bearing Plant Extracts on Common Lambsquarters (*Chenopodium album* L.)

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*Allelopathy can be regarded as a component of biological control in which plants are used to reduce development of other plants. Allelopathy refers to the direct or indirect chemical effects of one plant on the germination, growth or development of neighboring plants. The allelopathic effects of extracts of mint (Mentha piperita L.), thyme (Thymus vulgaris L.), rosemary (Rosmarinus officinalis L.), coriander (Coriandrum sativum L.) and sage (Salvia officinalis L.) on seed germination and some growth characteristics of common lambsquarters (Chenopodium album L.) were investigated. Aqueous extracts of aromatic plants at 0, 1, 2.5, 5, 10 and 20 % concentrations were applied to determine their inhibition effects on seed germination; seedling shoot and root length of C. album seed under laboratory conditions. The extracts of tested plant species caused inhibitory effects on seed germination and seedling length of C. album. Allolapaticity increased progressively with the increasing extract concentration. The results showed that total germination inhibition of C. album depended on the extract concentration; ranged from %13 to 100. The maximum inhibition (100%) rate for germination was obtained from the highest extract concentration for all test species. Extracts of mint, thyme, rosemary, coriander and sage could be used as alternatives herbicides.*

*Keywords: Allelopathy, plant extracts, seed germination, Chenopodium album*

Weeds are one of the major problems in world agriculture because they cause losses in crop yield [1]. Even with the intensive use of synthetic herbicides, weeds cause 10–30% crop losses, while without weed control crop losses could be 45–95%, depending on ecological and climatic conditions [2, 3]. Weed management is, therefore, a key factor for most the agricultural systems.

The use of synthetic chemical herbicides has increased in most of the cropping systems to control weeds [1]. To enhance productivity and crop protection, about 3 million tons of herbicides are used annually globally [4]. Although synthetic herbicides have been used successfully in weed control, they have adverse impacts on human health and the environment and considered today as a real problem. Intensive use of synthetic herbicides may promote the development of herbicide resistant weeds; enhance soil erosion, soil and groundwater contamination [5,1, 6]. Furthermore, there is high risk to drift herbicide to non-target plants and crops, and growing concern about herbicides residues. Due to the these mentioned concerns, alternative approaches are needed to supplement chemical weed management [1].

Researchers have focused on new potential bio-herbicides, having different and selective herbicidal mechanisms in comparison to their equivalent synthetic herbicides. Therefore, seeking suitable safe natural compounds as an alternative source is essential for weed management. One of the most practical alternatives to overcome human health and environmental problems is the use of natural compounds having allelopathic impacts on weeds in sustainable agriculture [3, 5, 7, 8]. Allelopathy is an interference mechanism which plants release chemical compounds which have adverse effects on other plants. Several plants have allelopathic release exudates from living tissues or by decomposition of plant residues which influence other plants in their surrounding vicinity [8 - 12]. The role of allelopathy as interactions of plant to plant and especially its potential for weed management in agriculture are considerably significant. Recently, allelopathy has received great attention due to their weed suppressing potential besides other multiple ecological roles [5, 8]. An allelochemical approach can also be taken in selecting plant species for extraction of phytotoxins. If a plant is known or suspected to be allelopathic, one should expect it to produce phytotoxic allelochemicals [13, 14].

Allelopathic phytotoxins may have novel target sites of action [8] that can be highly valuable in design of new synthetic herbicides. Allelochemical compounds extracted from plants may require less compulsory inspections for commercialization than the synthetic compounds, hence lowering the commercializing cost of the product. In some countries, synthetic compounds are either not allowed (e.g. organic farming) or long-term approval for commercialization. Lastly, despite the relatively low priority of natural products in herbicide discovery, there have been some major successes with natural products as herbicides [8].

Recently, use of allelochemicals extracted from plants has gained great attention as a feasible option for alternative weed management in sustainable agriculture systems [15-17]. In fact, numerous studies have been conducted to determine the potential use of allelochemicals extracted from higher plants for managing weeds under field conditions [7, 17-19].

One of the main objectives of studies on allelopathy is to gain insight into the mechanisms of weed/crop interactions and to utilize such information to improve the weed management strategies [20].

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Plants in Labiatae family possess essential oils, which could be utilized for managing weeds. Understanding of the plant biochemistry, physiology and chemistry of natural products have shown that the allelochemicals may be used for weed control to overcome the above problems associated with the herbicides. The bio herbicidal potential of essential oil bearing plants are well known [21]. Hydrocarbons and oxygenated compounds are two distinct groups of chemical constituents of pure essential oils that can be a primary source of potential allelochemicals [22, 23].

Common lambsquarters (*Chenopodium album* L.), found in many cultivated lands, is one of the most problematic weeds worldwide and it was considered one of the five most widely distributed weeds in the world [24]. The seeds of lambsquarters can germinate from mid-April through June. It is one of the first summer annuals to emerge in the spring and can be present before crops are planted. In the countries where common lambsquarters is widely controlled with synthetic herbicides, resistant common lambsquarters to photosystem II inhibitors (triazines), ALS inhibitors and suspected resistance to glyphosate was seen in the agricultural production systems [25- 27].

The objective of the present study was to investigate the effects of aqueous extracts of mint (*Mentha piperita* L.), rosemary (*Rosmarinus officinalis* L.), coriander (*Coriandrum sativum* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.), on germination inhibition of (*C. album* L.) seed of weeds.

## Experimental part

The tested plant species used in the current experiment were collected at flowering stage in 2013. Harvested plants were dried at room temperature. Dried samples were ground separately through a 40-mesh screen and stored at 5 °C until sued for bioassay. Common lambsquarters (*Chenopodium album* L.) seeds were collected in Erciyes University experimental fields Kayseri, Turkey in September 2013. Seeds from different plants were pooled and put in the storage at 5 °C.

### *Extracts preparation*

Aqueous extracts of tested plant species were prepared as described by Mennan et al. [1, 3]. To prepare 1, 2.5, 5, 10 and 20% concentration of plant extracts; ground samples (1, 2.5, 5, 10 and 20 g) were soaked in 100 mL distilled water for 24 h at 24 °C in a lighted room. The solutions were filtered through four layers of cheesecloth to remove debris and then centrifuged at 900 g for 4 h to remove fine plant debris. The supernatant was filtered through one layer of Whatman no. 42 filter paper. The solutions were filtered again through 0.2μm Nalgene filter to prevent microorganisms growth [1].

### *Bioassay test*

Various (0, 1, 2.5, 5, 10 and 20 g) concentrations were soaked in 100 mL distilled water and the solutions were obtained as described above. In bioassay, the effects of the extracts were determined on the seed germination, shout and root growth of common lambsquarters (*Chenopodium album*).

Before bioassays, seeds were surface sterilized in 10% commercial bleach (with 5% active chloride) solution, containing 0.1% Tween 80 for 10 min and rinsed several times with distilled water. Seeds were placed on moistened paper towels for 2 h. Thirty common lambsquarters (*C. album*) seeds were placed on filter paper in sterilized 9 cm dia Petri dishes. In each Petri dish, 7.5 mL of extract solution was added and distilled water was used as control. Petri dishes were covered by using parafilm. All Petri dishes were placed in a lighted growth chamber (16 h light 8 h dark) at 25 °C. Percent germination was determined and seedling shouts and root lengths were measured after 21$^{st}$ days of treatments.

Percent inhibition was calculated as:

$$[(\text{Control-Aqueous extracts})/\text{Control}] \times 100 \quad (1)$$

### *Determination of Total Phenolic Content*

Total phenolic content (TPC) of the aromatic plant samples was determined by a modified method [28]. Briefly, 0.5 mL of a properly diluted extract was mixed with 2.5 mL of 10 fold diluted Folin & Ciocalteu's phenol reagent and incubated for 1 min, before 2 mL of 7.5 % $Na_2CO_3$ was added. The mixture was allowed to stand for 30 min. The absorbance versus prepared blank was read at 765 nm (Shimadzu UV-1700 spectrophotometer, Shimadzu Corp, Kyoto, Japan). Gallic acid solutions (20–100 mg/L) were used for calibrations. The final results were expressed as mg gallic acid equivalent (GAE) per liter.

### *Statistical Analysis*

The experimental design for laboratory bioassays was a randomized design with four replications. Data from experiments mean values were separated based on the least significant difference (LSD) at 0.05 probability level. Analysis of variance was done for all data using a general linear model. A preliminary analysis of dose-response curves within aromatic plants showed that the data were best described with log-logistic curve [29]. Relative inhibitions of germination were analyzed using the four parameter log-logistic model where the D term was fixed at 100 [30] :

$$[y = D \times \exp - \exp(b \times \log(x) - \log(e))] \quad (2)$$

Where, *Y*: Response (e.g., seed germination *C. album*), *D:* Upper limit, *b:* Slope of the regression line, and x: Doses of extracts. EC50: Dose providing 50% response (50% reduction in seed germination, also known as the *inflection point*, I50, or EC50) were obtained from formula [2]. The analyses of dose–response curves were conducted using R and drc software packages as previously described [29, 31].

## Results and discussions

### *Total Phenolic Content of Aromatic Plants*

Total phenolic content (TPC) of the tested aromatic plant samples was determined. In general, TPC of plant extract increased with the increasing extract concentrations (fig. 1). While the highest TPCs were obtained from sage extracts, the lowest TPCs were acquired from coriander extracts. Following sage, the highest TPCs were obtained from mint, rosemary and thyme, respectively.

### *Germination*

The extract concentrations among tested plant species had different inhibitory effects on seed germination of *C. album*. The maximum inhibition (100%) rates of the tested species on germination were obtained from the highest extract concentrations (fig. 2). Total germination inhibition on the seeds of *C. album* varied between 13 and 100%, depending on the extract concentration.

The phytotoxicity of extracts was increased significantly with the increasing extract concentrations. The highest concentration of mint extract (10 and 20 g/100 mL) greatly

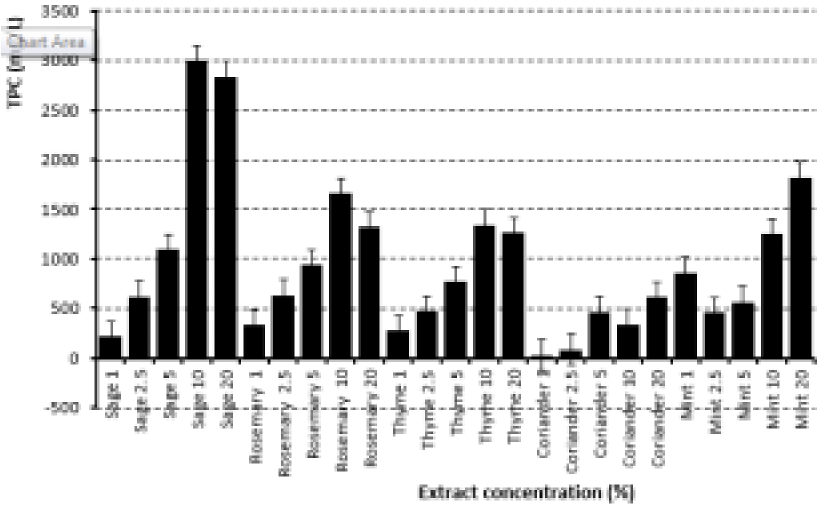


Fig. 1. Total phenolic content (TPC) of the tested aromatic plant samples (mg/L)

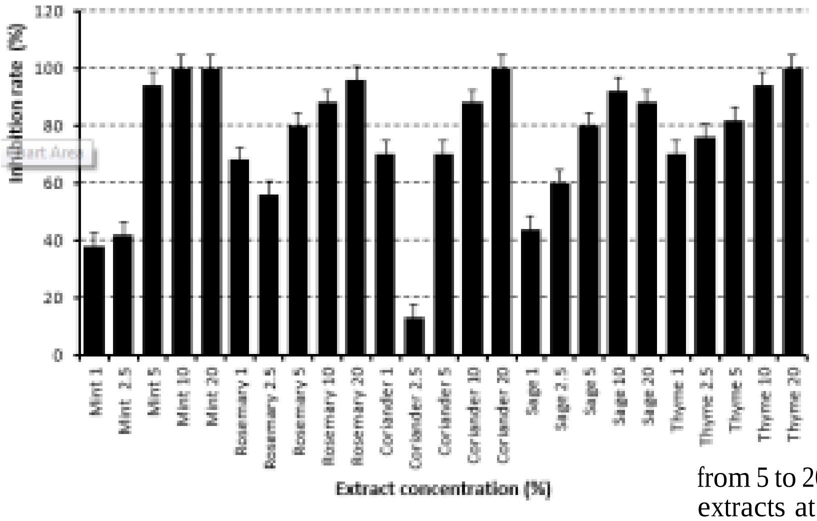


Fig. 2. The inhibitory effect of different concentration of aromatic plant extracts on germination of C. album. Bars represent standard error of means (P < 0.05)

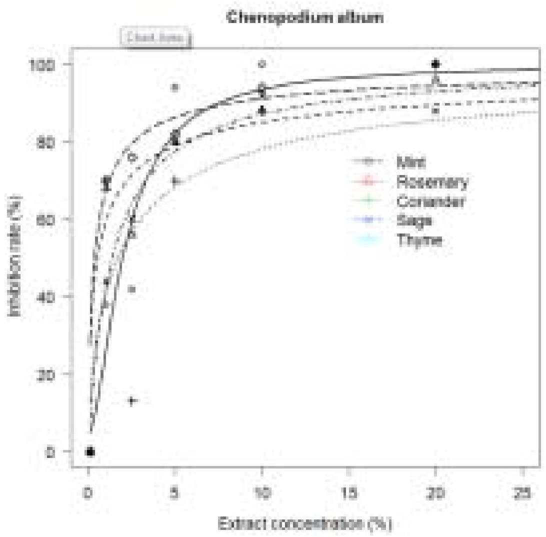


Fig. 3. Effect of aromatic plant extracts on germination of *C. album*. The lines were fitted accounting to eq. 2

inhibited the seed germination > 100% (fig. 3). However, germination rate of the lowest extract concentration of mint (1 g/100 mL) on *C. album* germination was 38%. The EC10, EC50, and EC90 of mint were 0.56, 2.07 and 7.71 g/100 mL, respectively (table 1). Dose-response curves were parallel however it was significantly different in EC10, and EC50. This parallelism is an indicator of similarity in the mode of action of an active compound. Similar trends in germination were observed for other aromatic plant extracts. The rosemary extract at 1 g/100 mL water inhibit the *C. album* germination 68%, the inhibition rate increased from 80% to 96% as the extract concentration increased from 5 to 20 g/100 mL (fig. 3). The application of coriander extracts at 2.5, 5, 10 and 20 g/100 mL caused 13, 70, 88, and 100% reductions in *C. album* germination, respectively. The inhibitory effect of sage and thyme extracts increased as the concentrations increased from 1 to 20 g/100 mL water (fig. 3). The EC10, EC50, and EC90 of thyme extracts were 0.02, 0.36 and 8.12 g/100 mL, respectively (table 1).

*Shoot Length*

The plant extract significantly reduced the *C. album* shoot length (fig.4). The highest concentrations of aromatic plant extracts showed the highest decrease rate in root length. While aqueous extracts of mint showed minimum suppressive effect; the aqueous extracts of thyme showed maximum suppressive effect on the root length of *C. album*. The EC10, EC50, and EC90 of thyme extracts were 0.002, 0.03 and 3.69 g/100 mL, respectively (table 2).

*Root Length*

The effects of plant aqueous extracts on root length were similar to shoot length. Plant extracts also significantly reduced the *C. album* root length and the highest inhibition rate on the root length was obtained from highest extract concentrations (fig.5). Maximum reduction of root length was observed from thyme extracts. The EC10, EC50, and EC90 of thyme extracts were 0.0006, 0.02 and 46.1 g/100 mL, respectively (table 3).

Development of natural compounds with herbicidal effects would help to decrease human health and environmental problems. In this point of view, natural herbicides may be effective, selective, biodegradable and less toxic to the environment. Certain plant species or their residues selectively inhibit development of particular species. This differential sensitivity observed in field, green house and laboratory experiments with residues, extracts and purified allelochemicals [32, 33 ].

| Extracts | Log-logistic parameters | | Effective concentration (EC) | | |
|---|---|---|---|---|---|
| | b | c | $EC_{10}$ | $EC_{50}$ | $EC_{90}$ |
| Mint | -1.67 | 4.40 | 0.56±0.3 | 2.07±0.5 | 7.71±1.99 |
| Rosemary | -0.59 | -0.1 | 0.01±0.02 | 0.52±0.34 | 21.73±15.4 |
| Coriander | -0.73 | 2.74 | 0.09±0.2 | 1.83±1.67 | 36.92±43.2 |
| Sage | -0.96 | -0.1 | 0.14±0.08 | 1.36±0.3 | 13.32±4.68 |
| Thyme | -0.7 | -0.1 | 0.02±0.03 | 0.36±0.26 | 8.12±4.78 |

Regression parameters were estimated using Equation 2.

**Table 1**
LOG-LOGISTIC REGRESSION PARAMETERS AND EFFECTIVE CONCENTRATION (EC10, EC50, AND EC90) OF AROMATIC PLANT EXTRACTS ON INHIBITION OF SEED GERMINATION OF *C. ALBUM*

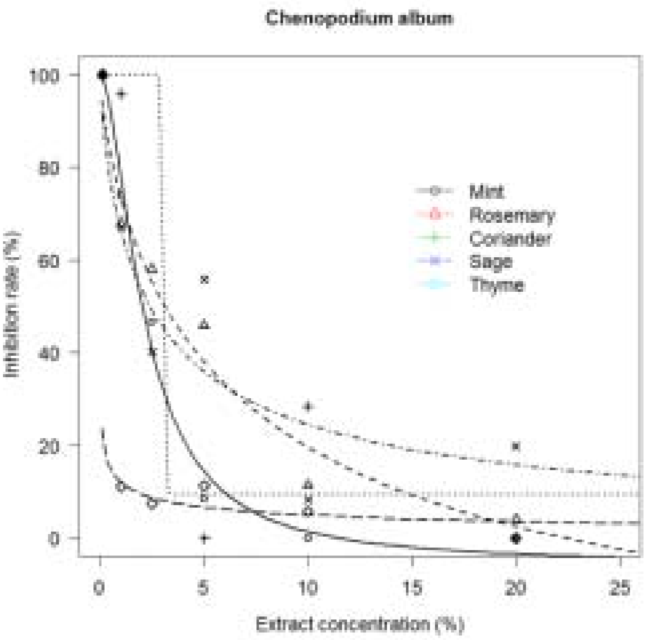


Fig. 4. Effect of aromatic plant extracts on shoot length of *C. album*. The lines were fitted accounting to eq. 2

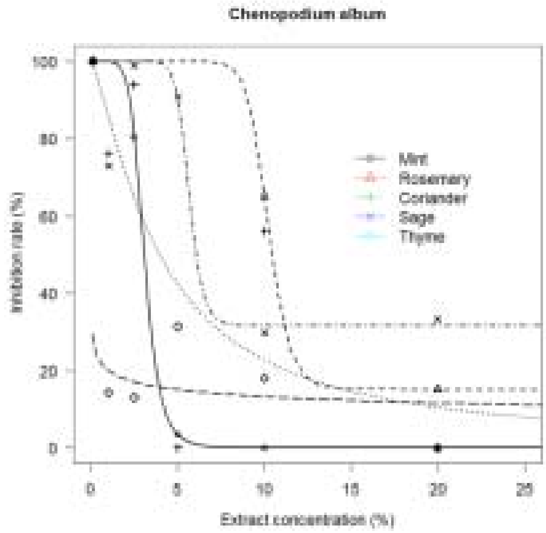


Fig. 5. Effect of aromatic plant extracts on root length of *C. album*. The lines were fitted accounting to eq. 2

| Extracts | Log-logistic parameters | | Effective concentration (EC) | | |
|---|---|---|---|---|---|
| | b | c | $EC_{10}$ | $EC_{50}$ | $EC_{90}$ |
| Mint | 1.73 | -5.53 | 0.6±0.25 | 2.14±0.4 | 7.67±3.76 |
| Rosemary | 0.75 | -41.46 | 0.38±0.2 | 6.96±9.3 | 128.2±334 |
| Coriander | 0.78 | 9.47 | 2.97±97.7 | 3.05±116 | 3.13±134.7 |
| Sage | 0.12 | 0.14 | 0.15±0.18 | 2.4±0.18 | 39.7±117.3 |
| Thyme | 0.31 | -2.69 | 0.002±0.01 | 0.03±0.01 | 3.69±10.5 |

Regression parameters were estimated using Equation 2.

**Table 2**
LOG-LOGISTIC REGRESSION PARAMETERS AND EFFECTIVE CONCENTRATION (EC10, EC50, AND EC90) OF AROMATIC PLANT EXTRACTS ON SHOOT LENGTH OF *C. ALBUM*

The results of the present research agree with most of the previous results obtained by other researchers, which emphasized that extracts of many plant species inhibited germination of many other weed seeds [34]. Also results from different parts of allopathic plants proved the inhibition effect on seed germination of some pasture plants [35, 36]. These findings also confirm that extracts of many plant species including aromatic herbs contain allelochemicals which have been reported to affect enzyme responsible for plant hormone synthesis, in addition to inhibition of nutrient and ion absorption by affecting plasma membrane permeability [37]. Essential oil bearing plants cause anatomical and physiological changes in plant seedlings which lead to accumulate lipid globules in the cytoplasm, reduce size of some cell organelles, possibly due to disruption of membranes, inhibition of DNA synthesis or alter membrane permeability [38-40].

In the present study, extracts of mint, thyme, sage, rosemary and coriander inhibited seed germination, seedling and root growth of *C. album* proportionally related with the concentration of the extracts. The results also indicated that most of the plant extract treatments have resulted in inhibition in radicle and plumule elongation. The effect of plant extract on radicle growth was studied by Lisanework and Michelson (1993) [41] and Khan et al., (2004) [42] with eucalyptus plant, Siddique et al., (2009) [43] with *Prosopis juliflora*. The result of the present work also supported the finding of above workers who found a significant decrease of radicle growth in many crop and garden plants.

Results of this study agree with what was found by Oudhia (1999) [44] where he found inhibition at growth in radicle and plumule of *Lathyrus sativus* by extracts from *C. gigantea*. El-Darier and Yousef (2000) [45] found a negative

**Table 3**
LOG-LOGISTIC REGRESSION PARAMETERS AND EFFECTIVE CONCENTRATION (EC10, EC50, AND EC90) OF AROMATIC PLANT EXTRACTS ON ROOT LENGTH OF *C. ALBUM*

| Extracts | Log-logistic parameters | | Effective concentration (EC) | | |
|---|---|---|---|---|---|
| | b | c | $EC_{10}$ | $EC_{50}$ | $EC_{90}$ |
| Mint | 6.86 | 0.03 | 2.23±0.2 | 3.07±0.4 | 4.23±1.17 |
| Rosemary | 17.0 | 15.14 | 8.98±19.6 | 10.22±4.45 | 11.62±35.37 |
| Coriander | 1.32 | 0.02 | 0.76±0.01 | 3.96±0.01 | 20.75±0.01 |
| Sage | 14,92 | 31,59 | 4.89±1.41 | 5.66±7.45 | 6.56±18.82 |
| Thyme | 0.22 | -0.11 | 0.0006±0.002 | 0.02±0.002 | 46.1±1499.4 |

Regression parameters were estimated using Equation 2.

relationship among different concentrations of alfalfa on plumule and radicle elongations of *Lepidium sativum*.

Radicle length of *C. procera* was relatively more sensitive to allelochemicals than that of plumule length. These results agree with the other studies reporting that water extracts of allelopathic plants had more pronounced effects on radicle growth than on plumule growth [46-48]. This is probable because the roots are the first part of plants to absorb the allelochemicals from the surroundings.

## Conclusions

Extracts of mint, thyme, sage, rosemary and coriander inhibited seed germination, seedling and root growth of *C. album* proportionally related with the concentration of the extracts.

A great deal of success on controlling *C. album* could be achieved only by the application of high concentration of aqueous extract of the tested species. The reduction of germination, shoot and root inhibition can be due to the effects of phenolic compounds which exist in aqueous extract of tested plants. The results of the current study showed that total seed germination inhibition of *C. album* varied between percent13 to 100, depending on the extract concentration. The maximum germination inhibition rate (100%) was obtained from the highest extract concentration of all test species. Further studies are needed to evaluate allelopathic activities of aqueous extracts of the tested species under field conditions.

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