# PHYTOCHEMICAL ANALYSIS OF PALIRUS SPINA CHRISTY

**Zakieh Beheshtinia**

*Department of Phytochemistry, Tehran_shargh payam noor University, Iran*

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## Abstract

T he Christ's thorn, scientifically named palirus spina Christy, mostly found in the Alborz foothills, was examined for this study. The present paper, thus, aims at the phytochemical analysis of palirus spina Christy. Collecting the plant from Chahar Dangeh, Sari, the limbs of the plant were separated, washed and dried, and were powdered using traditional methods. Using the Soxhlet apparatus, the essential oil of each plant limb was separately extracted in an *in vitro* environment by ethanol solvent. The extracts were dried using a rotary device and the solvent was removed. The dried extract of each limb was analyzed in the GC-Mass apparatus to identify the components and their percentage. GC-Mass analysis indicated that in addition to compounds that are widely used in industry and pharmacy, the plant has a high level of vitamin E and an anti-skin and prostate cancer compound known as Lupeol. The extracts underwent an antibacterial test to find their biological properties. The test indicated that, in comparison to the common antibiotics in the market, such as ampicillin, this is a good antibiotic or antibacterial compound that may be even stronger than the marketed antibiotics. In addition, the total phenol and flavonoid content, DPPh, and iron reduction tests were performed to find out the antioxidant properties of extracts. All of the tests showed that the plant is a strong antioxidant due to its vitamin E content and isomers. It is a natural anti-cancer medicine and is a good replacement for the chemical medicines on the market.

**Keywords:** palirus spina Christy, GC-Mass analysis, antibacterial test, antioxidant properties

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## Introduction

The science of medicine was established when human beings felt that they need plants to survive and maintain their health. From ancient times, medicinal plants have been used by human beings and have served as the foundation of traditional medicine in most civilizations (Qasemi, 2011). In ancient Iran, medicinal plants, as their name suggests, were used as drugs, antiseptic agents, and aromatizers (Kianmehr, 2007). Today, researchers extract the effective substances of medicinal plants through advanced procedures and devices to recognize their real values (Kianmehr, 2007). However, proper use of medicinal plants depends on precise scientific knowledge. Meanwhile, over time, due to the intervention of the ignorant and profiteers, myths and untrue facts have been intruding into traditional medicine, which should be discarded, and this separation requires experts and professionals so that the objectives could be achieved (Herbal medicines, Farshad Ebrahimpour).

Palirus Spina Christy is a deciduous shrub or short tree with a height of 2-3 m (sometimes 4m) with winter buds covered by double or triple scales with prickly wooden earrings. These prickly wooden earrings are usually 18mm in length and are straight in longer parts and cane-shaped in shorter ones. They have double-rowed alternative perfect or serrated leaves with a length of 2-3cm and a width of 3cm. Small, five-limbed male and female flowers are located on axial or sometimes terminal pistils with a depth of 3-4mm. The shield-shaped calyxes have wide triangular lobes 2 mm long. The petals are small, greenish-yellowish, and rotating. The 2 or 3- gynoecium ovary is attached to the receptacle. The plant has a woody fruit with 2 to 3 carpels each with a smooth stone. The fruit has a semi-spherical shape surrounded by wide membranous arms.

This plant has been used in many regions, where the wood and stem of the plant were taken as an antipyretic. The plant was known and used from ancient times by local and rural people. It has been used as an astringent, nutritious, and diuretic agent and its roots and leaves were used to treat diarrhea. This prickly shrub, with its medicinal fruit, grows in various parts of Mazandaran Province in the forest habitats and pastures of mountainous and often dry slopes, especially along roads and ruined areas in low heights of Haraz, Chalous valley, Kojoor valley, Talar valley, and in the ruined parts of the plains and sometimes next to farms and forest parks and at a height of 25 to 1000m, and sometimes 1500m, from the sea level.

This prickly plant was collected during the fall from Chahar Dangeh (town of Kiasar), southern Sari, and all of the plant limbs, including root, stem, the tip of the stem, thorn, leave, flower and fruit were tested. Due to its thorns all over the plant, people used the plant from long ago in their farms to protect their lands against animals. This caused ignorance toward the plant's medicinal and other properties. Considering that all parts of the plant have been taken into account in this thesis, its displantation was very difficult due to its thorns and strong and deep root. So, this study aims at the phytochemical analysis of palirus spina Christy.

## Materials and methods

Palirus Spina Christy was displanted along with its roots from one of the Northern Kiasar farms (southern Mazandaran Province) in a traditional way and using a shovel. Using an ax, its thick parts were cut into pieces, and the other parts were separated by hand. Each part was placed separately in a special container. The plant was washed gently with tap water so that the dirt is removed. It was dried under shade on a white, clean cloth at room temperature. The thin and soft parts of the plant such as stems, tips, thorns, leaves, flowers, and fruits were powdered using a domestic mill and the thicker pars using an industrial mill.

To extract the essential oil, a filter paper was shaped like a cylinder (closed on both ends). A specific amount of each part of the plant was poured into a cylinder and placed in the Soxhlet apparatus. Then half of the soxhlet balloon was filled with ethanol and place in an electrically heated shuff balloon. The ethanol boiling temperature was applied (78°C). The mixture was heated for 6 h and the refluxing process was repeated 17 times.

The essential oil from most parts of the plant was greenish (dark – light) except that of roots and thorns, which were brown in color. The extracts were placed on a rotary device and the solvent was removed. The so-called dried extract was poured into a capped glass container. To analyze the compounds by the GC-MS device and to perform other tests, the extracts were sent to the laboratory.

**Figure 1. Grounded plant**

## Findings

Extracts were obtained from seven parts of the plant. The antioxidant and antibacterial properties of each limb were examined through the GC-Mass apparatus.

## GC-Mass results analysis

The results of the GC-Mass test indicated that the plant limbs, especially roots, flowers, and leaves include a high amount of vitamin E. it should be noted that vitamin E is a strong antioxidant that is essential for the body. Anti-cancer compounds such as beta-sitosterol have been observed in both stems and leaves, beta-tocopherol in flowers, and a compound with the commercial name Lupeol, which has many potential medicinal properties. Compounds such as pyrogallol and linoleic acid, which have been found in the roots, are also widely used in the cosmetics industry.

The compound that attracted our interest in this study was genol. People living in the area where this plant was harvested knew that the grounded form of the upper limbs of this plant could be used as a poultice to relieve pain, especially foot pain. The results of the experiment showed that this is probably due to the presence of genol in the stem tips, which are used today as a hypnotic and anesthetic. It should also be noted that this compound has been only found in the stem tip and not in other plant limbs.

**Antibacterial analysis**

The effect of the increase in the extract volume on the bacterial growth control was evaluated. The studied bacteria were both gram-positive and gram-negative ones, including:

A) Gram-negative: E. coli (1) and Klebsiella pneumoniae (2)

B) Gram-positive: Staphylococcus aureus (3) and Bacillus subtilis (4)

The extracts used in the antibacterial examination were ethanol extracts obtained through the Soxhlet.

As shown in Fig. 1-3, the effect of the increase in the amount of plant root extract from 12.5 ml to 100 ml is clear on both gram-positive and gram-negative bacteria.

a. Inhibitory effect of the lowest amount on gram-negative bacteria EC = 6 and KP = 6 and inhibitory effect of the highest amount on gram-negative bacteria EC = 20 and KP = 15

b. Inhibitory effect of the lowest amount on gram-negative bacteria StA=10 and BS = 6 and inhibitory effect of the highest amount on gram-negative bacteria StA= 21 and BS = 18.

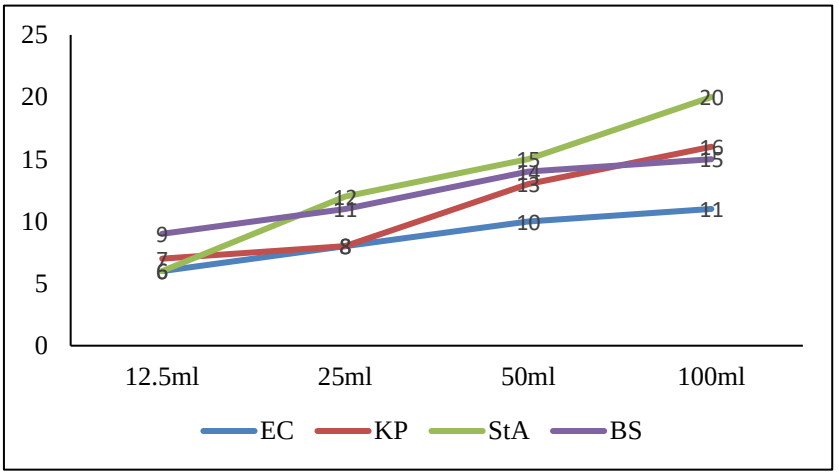


**Figure 1: Inhibition zone diameter of root ethanolic extract**

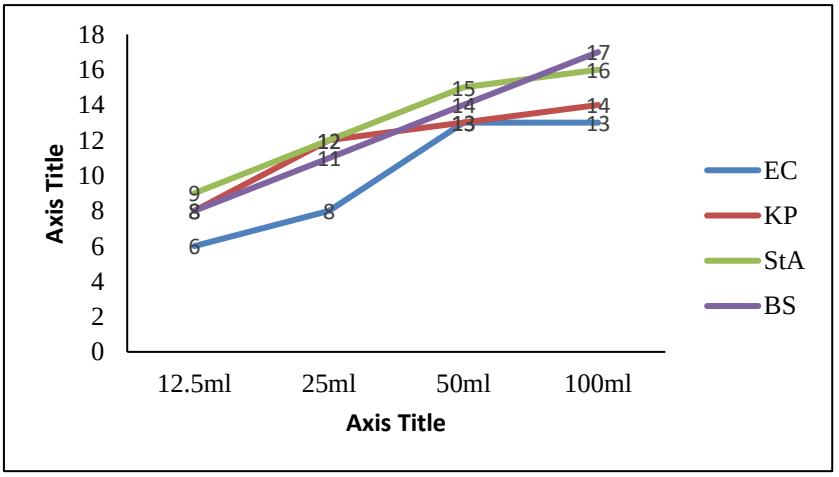


**Figure 2: Inhibition zone diameter of stem ethanolic extract**

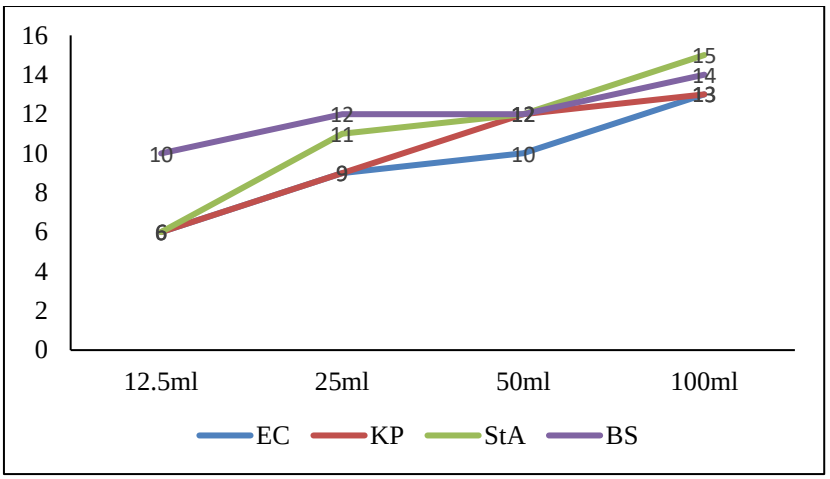


**Figure 3: Inhibition zone diameter of stem tip ethanolic extract**

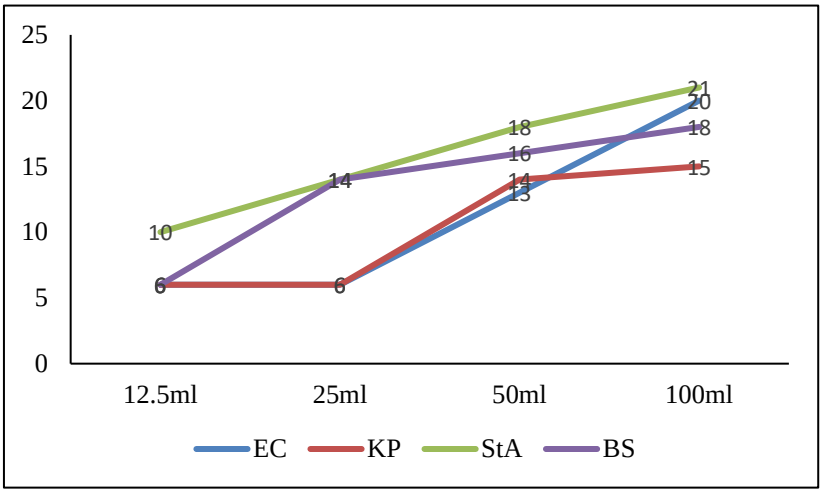


**Figure 4: Inhibition zone diameter of thorn ethanolic extract**

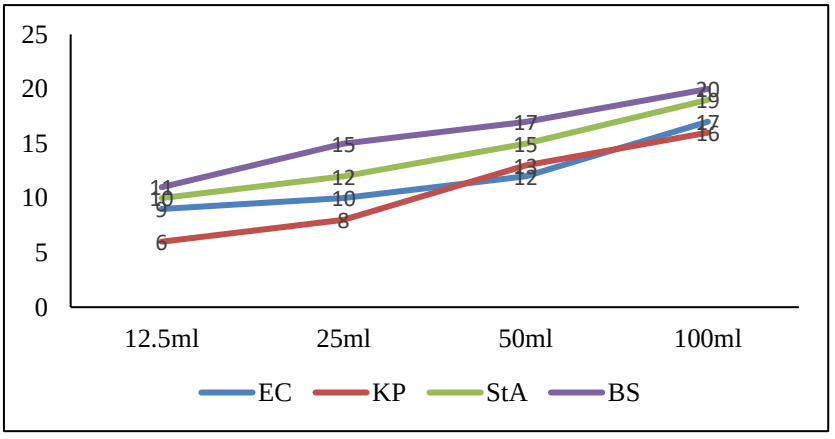


**Figure 5: Inhibition zone diameter of leaf ethanolic extract**

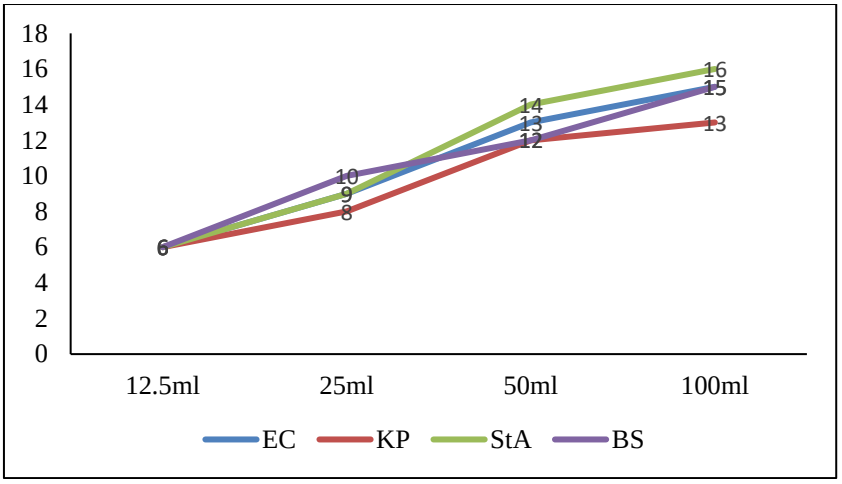


**Figure 6: Inhibition zone diameter of flower ethanolic extract**

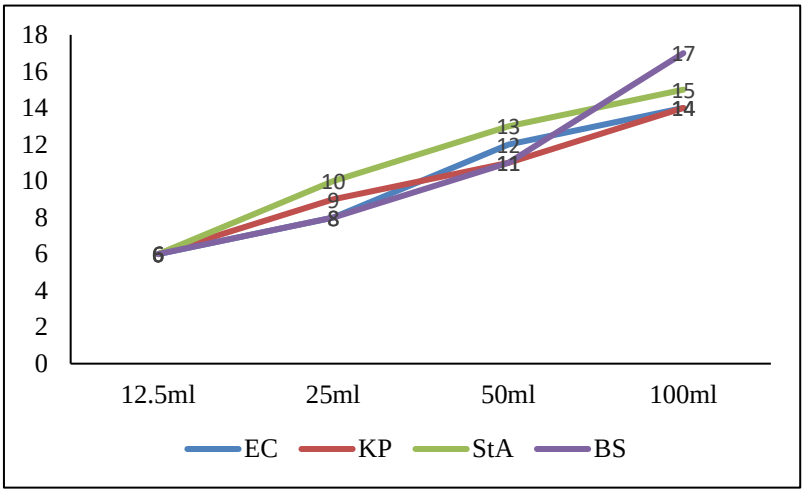


**Figure 7: Inhibition zone diameter of fruit ethanolic extract**

As shown in the diagrams, some parts of this plant could serve as a strong antibiotic comparative to the common antibiotics in the market such as ampicillin. For example, at the concentration of 100, ampicillin has an inhibitory rate of 22mm against Bacillus, and the extract of the leaves and flowers of this plant has an inhibitory rate of 20mm against the bacteria. Also, at the concentration of 100, Streptomycin has an inhibitory rate of 14mm against E-coli, while the leaf extract of this plant had an inhibitory rate of 20mm. In addition, at the concentration of 100, the aforementioned antibiotic has an inhibitory rate of 11mm against Staphylococcus aureus, while the leaf extract of this plant had almost twice the inhibitory rate, i.e. 22mm. This test was also performed in comparison with other commonly used antioxidants. It was concluded that this plant can induce an inhibitory effect close to, or even more than, some antibiotics, and this means that the plant is a natural replacement for some chemical antibiotics.

## Antioxidant properties

The total phenol and flavonoid content, DPPh, and iron reduction tests were used to investigate the antioxidant properties of the plant. Galic acid and Coersin flavonoid were used as standard. The absorption results have been provided in Tables (1) and (2).

**Table 1: Total phenol content with standard gallic acid**

| Gallic acid concentration (mg/ml) | 25 | 50 | 100 | 200 | 400 |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Absorption rate | 0.1988 | 0.3328 | 0.6018 | 1.1438 | 2.228 |

**Table 2: Total phenol content with standard Coersin flavonoid**

| Coersin concentration (mg/ml) | 25 | 50 | 100 | 200 | 400 |
|---|---|---|---|---|---|
| Absorption rate | 0.0994 | 0.186 | 0.383 | 0.798 | 1.585 |

Finally, the absorption rate of phenol and flavonoid of each extract is provided in the following diagram:

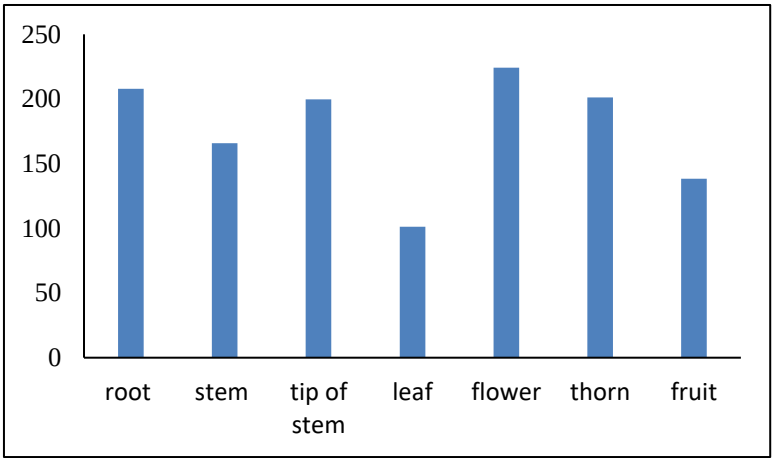


**Figure 8. Total phenol content at 400 mg/ml**

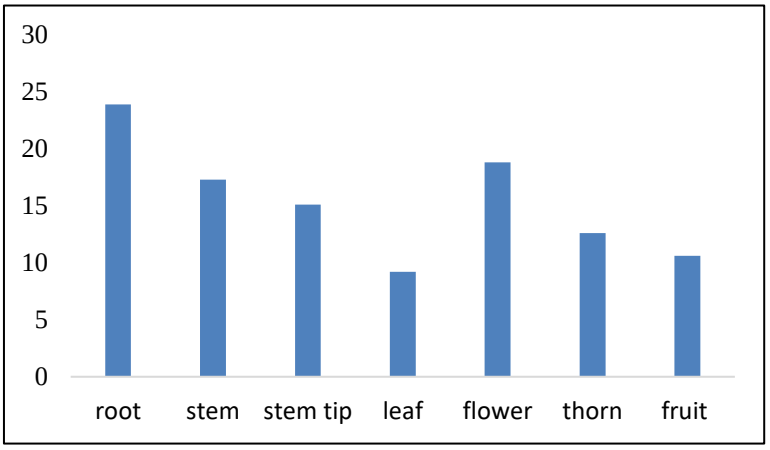


**Figure 9. Total flavonoid content at 400 mg/ml**

Leaf <fruit <stem <stem tip <thorn <root <flower (phenol)

Leaf <fruit <thorn <stem tip <stem <flower <root (flavonoid)

So, it can be concluded that overall, the flower and root could be good antioxidants with their phenol and flavonoid content.

The DPPH methodology is investigated in the following. The DPPH compound is a cancerous agent in itself as it is a free radical. If the extract could inhibit this free radical, it can induce anti-cancer effects. In this section, we have investigated the inhibition rate and mean absorption of each extract. As observed in Table 3 and Fig. 9, the DPPH inhibition rate is related to the root extract at the maximum concentration of 73.07% and the minimum concentration of 14.07%, which are far away from the control maximum and minimum concentrations.

**Table 3: Inhibition rate and mean DPPH absorption of root extract**

| Concentration (mg / ml) | 50 | 100 | 200 | 400 | 800 |
|---|---|---|---|---|---|
| Inhibition rate | 14.07 | 20.75 | 33.79 | 43.47 | 73.07 |
| Mean absorption | 0.687 | 0.685 | 0.681 | 0.493 | 0.243 |

**Table 4: Inhibition rate and mean DPPH absorption of stem extract**

| Concentration (mg / ml) | 50 | 100 | 200 | 400 | 800 |
|---|---|---|---|---|---|
| Inhibition rate | 12.03 | 18.31 | 40.81 | 42.12 | 54.65 |
| Mean absorption | 0.592 | 0.567 | 0.401 | 0.383 | 0.302 |

**Table 5: Inhibition rate and mean DPPH absorption of stem tip extract**

| Concentration (mg / ml) | 50 | 100 | 200 | 400 | 800 |
|---|---|---|---|---|---|
| Inhibition rate | 7.23 | 22.11 | 25.82 | 32.52 | 41.21 |
| Mean absorption | 0.617 | 0.518 | 0.502 | 0.477 | 0.392 |

**Table6: Inhibition rate and mean DPPH absorption of leaf extract**

| Concentration (mg / ml) | 50 | 100 | 200 | 400 | 800 |
|---|---|---|---|---|---|
| Inhibition rate | 6.07 | 14.93 | 20.2 | 27.63 | 33.17 |
| Mean absorption | 0.493 | 0.533 | 0.593 | 0.633 | 0.683 |

**Table 7: Inhibition rate and mean DPPH absorption of flower extract**

| Concentration (mg / ml) | 50 | 100 | 200 | 400 | 800 |
|---|---|---|---|---|---|
| Inhibition rate | 37.4 | 71 | 82 | 86.03 | 86.3 |
| Mean absorption | 0.483 | 0.221 | 0.133 | 0.108 | 0.103 |

**Table 8: Inhibition rate and mean DPPH absorption of thorn extract**

| Concentration (mg / ml) | 50 | 100 | 200 | 400 | 800 |
|---|---|---|---|---|---|
| Inhibition rate | 19.63 | 30.09 | 61.53 | 84.73 | 86 |
| Mean absorption | 0.624 | 0.543 | 0.293 | 0.113 | 0.103 |

**Table 9: Inhibition rate and mean DPPH absorption of fruit extract**

| Concentration (mg / ml) | 50 | 100 | 200 | 400 | 800 |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Inhibition rate | 21.43 | 28.12 | 53.51 | 65 | 78 |
| Mean absorption | 0.527 | 0.481 | 0.311 | 0.237 | 0.154 |

The results showed that the rate of DPPH free radical trapping is about 95% at a concentration of 800 and this indicated that the rate of DPPH trapping by root organs is about 74%, leaves 86%, flowers 86%, and fruit 78%. This shows that this plant can be a strong inhibitor of free radicals in the body and thus an anti-cancer agent.

**Reduction methodology**

Iron (III) is a carcinogen agent in itself. To become harmless, it should be converted to iron (II). In this section, the reduction power of iron (III) by the extracts of the plant was evaluated. The considered standard is vitamin C.

**Table 10: Reduction power of ascorbic acid as standard**

| Mean absorption (Mean ± SD) | Concentration (mg / ml) |
|---|---|
| 2.464 ± 0.2 | 800 |
| 2.172 ± 0.1 | 400 |
| 2.064 ± 0.3 | 200 |
| 1.982 ± 0.1 | 100 |
| 1.954 ± 0.1 | 50 |

In Fig. 10, it is observed that the reducing power of iron (III) by the flower was higher than other limbs.

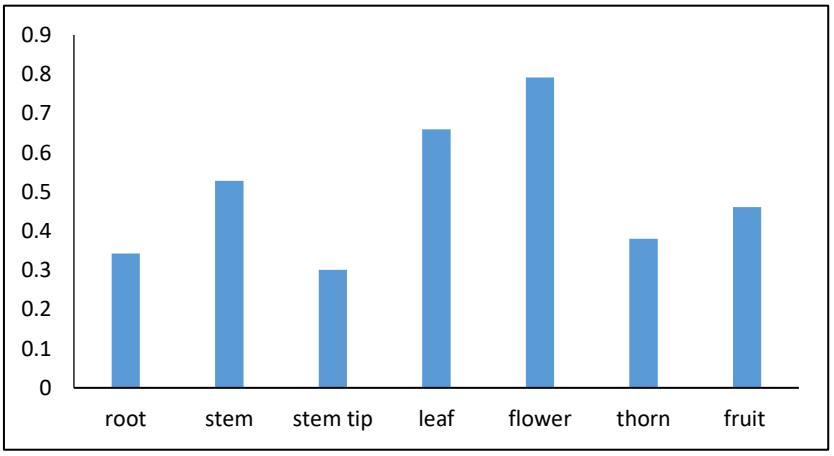


**Figure 10: Reduction of iron (III) by extracts at a concentration of 50 mg/l**

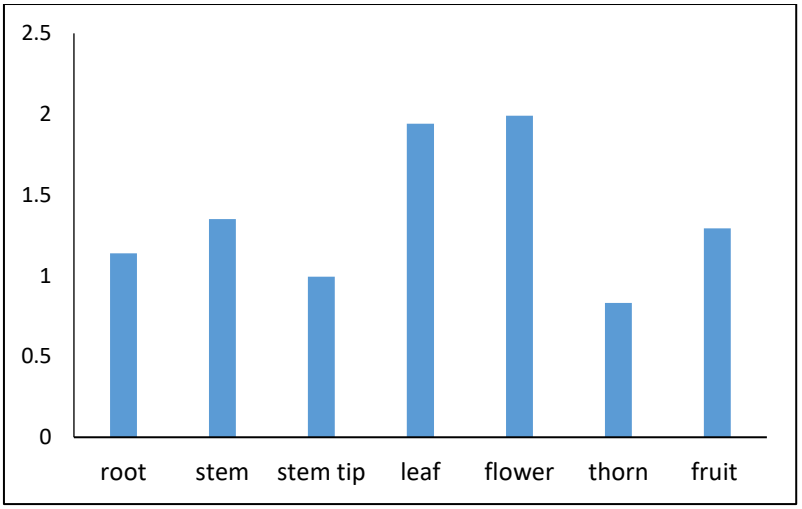


**Figure 11: Reduction of iron (III) by extracts at a concentration of 800 mg/l**

Iron test for different plant limbs indicates that, at the highest concentration and in comparison with ascorbic acid, most of them do not have a high absorption rate, except flower which showed an absorption rate of about 2 at the concentration of 800 compared to the absorption of vitamins C at the same concentration, which is 2.44. This limb probably had a good reduction power due to the presence of a large amount of beta-tocopherol (area = 54) and vitamin E (area = 550).

## Conclusion

The present study aimed at the phytochemical analysis of palirus spina Christy. The GC-Mass analysis indicated that some plant limbs such as flowers and leaves include large amounts of vitamin E. Vitamin E acts as a good anti-oxidant and is essential for the body. Also, there are anti-cancer compounds such as beta-sitosterol in both stem and leaf, betatocopherol in flowers, and a compound named Lupeol with many potential medicinal properties in the plant. Antibacterial and antioxidant experiments indicate that this plant stronger than, and a good replacement for, chemical antibacterial and antioxidant compounds I the market.

The results also showed that some parts of this plant could serve as a strong antibiotic comparative to the common antibiotics in the market such as ampicillin. For example, at the concentration of 100, ampicillin has an inhibitory rate of 22mm against Bacillus, and the extract of the leaves and flowers of this plant has an inhibitory rate of 20mm against the bacteria. Also, at the concentration of 100, Streptomycin has an inhibitory rate of 14mm against E-coli, while the leaf extract of this plant had an inhibitory rate of 20mm. In addition, at the concentration of 100, the aforementioned antibiotic has an inhibitory rate of 11mm against Staphylococcus aureus, while the leaf extract of this plant had almost twice the inhibitory rate, i.e. 22mm. This test was also performed in comparison with other commonly used antioxidants. It was concluded that this plant can induce an inhibitory effect close to, or even more than, some antibiotics, and this means that the plant is a natural replacement for some chemical antibiotics.

It can be concluded that, overall, the flower and root could be good antioxidants with their high amounts of phenol and flavonoid contents. It was indicated that the rate of DPPH free radical trapping is about 95% at a concentration of 800 and this indicated that the rate of DPPH trapping by root organs is about 74%, leaves 86%, flowers 86%, and fruit 78%. This shows that this plant can be a strong inhibitor of free radicals in the body and thus an anti-cancer agent.

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