

# Promotion Saudi-customs Using *Coleus forskohlii* L., Via In-Vitro Water-extract on Human-pathogenic-bacteria, Ranyah-region

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## Abstract

**Background:** *Coleus forskohlii* plant called in KSA (Al-Shar or Aspane Zattar), it discovery nearly at western south area, KSA. It contained flavonoids, alkaloids, tannins, terpenoids, steroids, saponins, and sugars. The essential oil had crucial result against human-pathogenic-bacteria as *Bacillus Spp.*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

**The aim:** It was the first time in Ranyah-region, KSA for proving and supporting the ancient Saudi-customs using *Coleus forskohlii* plant boiled-water-extract to protect person-health from human-bacterial-diseases and to consider as medicinal plant that easy getting, cheap and grows mostly in the southwestern region, KSA.

**Materials and Methods:** *Coleus forskohlii* plant were collected from Ranyah-region, KSA, to powder obtaining. The identified human-pathogenic-bacteria were obtained via the laboratory from diagnosed infected human cases. The bacterial suspension were added to boiled-water-extract, were followed up the bacterial growth.

**Results:** The average dry cell weight estimation showed *Pseudomonas aeruginosa* stayed for 50 hour, *Klebsiella pneumoniae* 45 hour and *Escherichia coli* 40 hour. *Staphylococcus aureus* remained alive for 35 hour, *Bacillus Spp.*, 30 hour. The average membrane filter estimation was found *Escherichia coli* did not give a large number of colonies at 40 hours, *Klebsiella pneumoniae* stopped producing colonies till 45 hours and *Pseudomonas aeruginosa* persisted until 50 hour.

**Conclusions:** It was concluded that the *Coleus forskohlii* plant boiled-water-extract eliminated human-pathogenic-bacteria, as the ancient Saudi-customs were based on its use for the person-health and its adoption as a medicinal plant.

**Recommendations:** It was recommend that the plant considering as a Saudi medicinal plant and using daily to maintain the person-health.

**Key words:** *coleus forskohlii*, KSA, (Al-Shar or Aspane Zattar), human-pathogenic-bacteria

## Introduction

*Coleus forskohlii* plant is a public earthy medicinal plant from *Coleus* spp., discovery in Asia, Africa, Australia, and Pacific Islands [1]. Its phytochemical crude extracts contain saponins, quinone, flavonoids, cardiac glycoside, tannins, phenols, terpenoids, steroids, alkaloids, and coumarins. That has antibacterial against human-pathogenic-bacteria as *Bacillus Spp.*, *cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. As well it useful in the drug create antibacterial action [1]. The leaves extract has total phenolic and flavonoid content, has antibacterial activity against human-pathogenic-bacteria as *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC 29213) and *Klebsiella*

*pneumoniae* (MTCC39), so it has the value of medicinal plants [2]. It's called in KSA (Al-Shar or Aspane Zattar), it discovery nearly at western south area, KSA, the fresh leaves were collected from Shada Mountain, Al-Baha area at (11-12) / 2019. The extracted essential oil was screened *in-vitro* for antibacterial activity against human-pathogenic-bacteria that contained flavonoids, alkaloids, tannins, terpenoids, steroids, saponins, and sugars. That was thymol (52.02 %),  $\gamma$ terpinene (18.70 %), o-cymene (12.73 %), 2-Undecanone (2.57 %), 6-methyl-2- heptanol, acetate (1.50 %), 4-carene (1.47 %) and caryophyllene oxide (1.36 %) [3]. There essential oil had crucial result against human-pathogenic-bacteria as *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC

27853). The essential oil extracted loaded phytochemical of pharmaceutical value as a broad-spectrum antibacterial factor so suggested as medicinal plants [3]. The aim was to prove this plant as the simple medicinal plant that easy getting, cheap and grows mostly in the southwestern region, KSA.

**Materials and Methods**

- *Coleus forskohlii* plant in Ranyah-region is famous presence in the valley and mountains, this plant was collected at the time of flowering and was graded by the "Botany Department". The samples were washed with distilled water, was dried for five days, and was milled in a sterile mixer to obtain the powder. So was added 5 gram powder in 25 mL of double distilled water, then was boiled for 30 min given boiled-water-extract, that was left at 4°C for 72 hour [4].
- The identified human-pathogenic-bacteria were obtained via the laboratory from diagnosed infected human cases. The obtained bacteria were sub-cultured onto Mueller-Hinton agar for 24 hour, then the colonies were grown in Mueller-Hinton broth as bacterial suspension matching the 0.5 McFarland turbidity standard equal

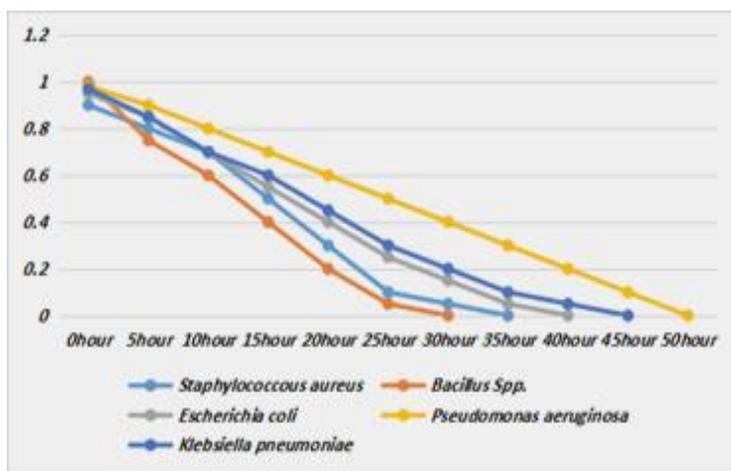
(1.5X10<sup>8</sup> CFU / mL) [5].

- The bacterial suspension 5 ml were added to 22 test tubes for each bacterial strain, the boiled-water-extract 5 ml were added to each test tube. The all test tubes were incubated at 35-37°C, then the test tubes were followed up from the experiment start and every five hours [6].
- Dry cell weight estimation: The bacterial cells were washed several times with sterile distilled water, the cells were dried and were weighed [7].
- Membrane filter estimation: The bacterial suspension were passed through a thin membrane filter, then filtration bacteria were present on the filter surface. The filters were transferred to the Mueller-Hinton agar, were incubated at 37°C for 24 hour, the colonies growth from the bacteria on the filter's surface were recorded [8].
- The data had recorded and had treated by "Simple Excel Sheet" [9].

**Results and Discussion**

Item	Time by hour											
	0 hour	5 hour	10 hour	15 hour	20 hour	25 hour	30 hour	35 hour	40 hour	45 hour	50 hour	
<b>Weight by Gram</b>												
<b>Staphylococcus aureus</b>	0.90	0.80	0.70	0.50	0.30	0.10	0.05	0.00				
<b>Bacillus Spp.</b>	1.00	0.75	0.60	0.40	0.20	0.05	0.00					
<b>Escherichia coli</b>	0.95	0.85	0.70	0.55	0.40	0.25	0.15	0.05	0.00			
<b>Pseudomonas aeruginosa</b>	0.98	0.90	0.80	0.70	0.60	0.50	0.40	0.30	0.20	0.10	0.00	
<b>Klebsiella pneumoniae</b>	0.97	0.85	0.70	0.60	0.45	0.30	0.20	0.10	0.05	0.00		

**Table 1:** The average dry cell weight estimation during the In-Vitro boiled-water-extract on human-pathogenic-bacteria



**Graph 1:** The average dry cell weight estimation during the In-Vitro boiled-water-extract on human-pathogenic-bacteria

Table 1 and graph 1 discovered the average dry cell weight estimation during the In-Vitro boiled-water-extract on human-pathogenic-bacteria, the experiment was conducted using the bacterial isolates cause serious and infectious diseases to humans and affect the person-health called human-pathogenic-bacteria. The experiment proved through the dry cell weight estimation from weight by Gram, which is the amount of bacterial living cells that continued to struggle with the boiled-water-extract and live longer [1-3]. Through the experiment, that found the Gram-negative bacteria are more resistant to the boiled-water-extract than Gram-positive

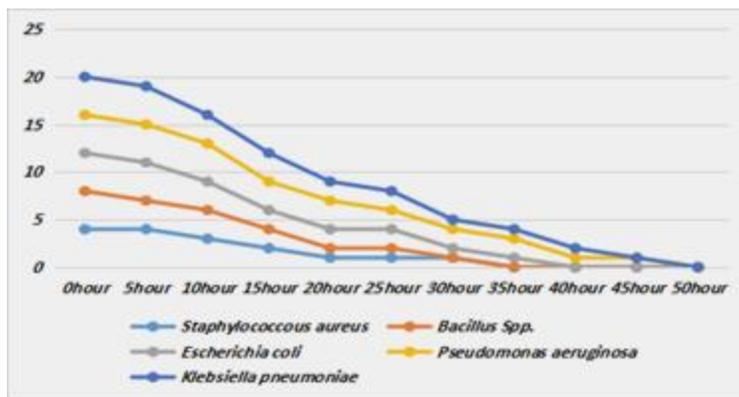
bacteria. As well that found the human-pathogenic-bacteria infect humans and cause many diseases which affect the person-health. *Pseudomonas aeruginosa* stayed for more than two days (50 hour) in order to be eliminated, followed by *Klebsiella pneumoniae* lasted for about two days (45 hour), then the last was *Escherichia coli* lasted for less than two days (40 hour) [1-3]. For Gram-positive bacteria, they were eliminated by weighing the cells that during more than a day, the strongest of them was *Staphylococcus aureus* that remained alive for about a day and a half (35 hour), then the following was *Bacillus Spp.*, remained about (30 hour) [1-

3]. From here it was clear that the components of the *Coleus forskohlii* plant that were boiled-water-extracted was the easiest method used and may be used in cooked foods and thus allow the opportunity for the components include “flavonoids, alkaloids, tannins, terpenoids, steroids, saponins, and sugars”; to be extracted from the *Coleus forskohlii* plant and help in the elimination of human-pathogenic-bacteria [1-3]. This proved that the use of the boiled-water-extract must be continued to eliminate human-pathogenic-bacteria, which need about two days or more

to eliminate them and preserve the person-health [1-3]. This supported the ancient Saudi-customs of using the *Coleus forskohlii* plant and proved that the *Coleus forskohlii* plant is considered as simple medicinal plant. It is necessary to follow the research of the “Pharmacy Department” to evaluate the components and healthy quantities that can be used by individuals to protect the person-health and thus be used in healthy ways without causing harm to the individual [1-3].

Item	Time by hour											
	0 hour	5 hour	10 hour	15 hour	20 hour	25 hour	30 hour	35 hour	40 hour	45 hour	50 hour	
<b>Colony count marks</b>												
<b>Staphylococcus aureus</b>	4	4	3	2	1	1	1	0				
<b>Bacillus Spp.</b>	4	3	3	2	1	1	0					
<b>Escherichia coli</b>	4	4	3	2	2	2	1	1	0			
<b>Pseudomonas aeruginosa</b>	4	4	4	3	3	2	2	2	1	1	0	
<b>Klebsiella pneumoniae</b>	4	4	3	3	2	2	1	1	1	0		

**Table 2:** The average membrane filter estimation during the In-Vitro boiled-water-extract on human-pathogenic-bacteria



**Graph 2:** The average membrane filter estimation during the In-Vitro boiled-water-extract on human-pathogenic-bacteria

Table 2 and graph 2 discovered the average membrane filter estimation during the *In-Vitro* boiled-water-extract on human-pathogenic-bacteria, through the experiment to estimate the growth through filtering and the use of filter paper for the colony count marks of bacteria, the growth of the colony indicated the presence of a living cell. Through the results colony count marks of the experiment, it was found that the growth of Gram-negative bacteria is more than Gram-positive, and this was through the effect of the components of *Coleus forskohlii* plant include “flavonoids, alkaloids, tannins, terpenoids, steroids, saponins, and sugars”; to eliminate cells and thus not to grow colonies [1-3]. It was found from Gram-negative *Escherichia coli* that the most effective by boiled-water-extract, as it did not give a large number of colonies except for a very short time, which means that the colonies were eliminated through the elimination of the living cell by the components of the *Coleus forskohlii* plant, and this was done at 40 hours. Next came the most powerful *Klebsiella pneumoniae* stopped producing colonies at 45 hours, the more strongest was *Pseudomonas aeruginosa* persisted until the end of the experiment and produced colonies until 50 hour, which indicated their ability to confront the boiled-water-extract and continue to produce colonies [1-3]. For Gram-positive bacteria *Bacillus Spp.* was the least capable of producing colonies and lasted for 30 hours, followed by *Staphylococcus aureus* the most capable of producing colonies, which

lasted for 35 hours [1-3]. From the experiments that were done, the effect of the extract on the *Coleus forskohlii* plant contents is consistent with the previous experience in the production of bacterial live cells and colonies [1-3]. Bacteria were arranged according to the most able of them to resist exposure to the boiled-water-extract and performed the two experiments, that through the first more powerful Gram-negative bacteria containing (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*); respectively, and then Gram-positive (*Staphylococcus aureus* and *Bacillus Spp.*); respectively [1-3]. The result indicates the importance of the *Coleus forskohlii* plant boiled-water-extract components and their effect on human-pathogenic-bacteria. Through the two experiments, it supported the ancient Saudi-customs of using the *Coleus forskohlii* plant boiled-water-extract as a drink or used in cooking and helps protect the person-health. Therefore, it was found that the *Coleus forskohlii* plant is one of the medicinal plants that may be used medically for treatment and prevention of human-pathogenic-bacteria to protect the person-health through the responsible health authorities [1-3].

### Conclusion

It was concluded that the *Coleus forskohlii* plant found as a result of dealing with the boiled-water-extract in laboratory experiments, the extent of the ability to eliminate human-pathogenic-bacteria, but it varies

according to the extent of the bacteria's resistance to the *Coleus forskohlii* plant boiled-water-extract components, as the ancient Saudi-customs were based on its use for the person-health and its adoption as a medicinal plant.

### Recommendation

It was recommend that the *Coleus forskohlii* plant considering as a Saudi medicinal plant and using the boiled-water-extract daily to maintain the person-health, but to a known extent, and the "General practitioner Doctor" should be consulted for cases that could give side effects.

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