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A Comparative Study of the Antimicrobial Properties of the Phenolic Extracts from " *Citrullus Colocythis* " Growing in Oued Souf

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Abstract

The Objective: The aim of this study was to analyze the qualitative and quantitative phytochemicals, assess the properties of antibacterial in the laboratory and identify biologically active compounds in extracts *Citrullus Colocythis* Collected from ElOued in the region Hassani and Hamraya. **Method:** A quantitative, quantitative, and in vitro quantitative qualitative analysis was performed using standard procedures. Biologically active compounds were analyzed**. Results:** Through the results, we deduce that: The phenolic extract for the region of Hassani was better than Hamraya region where it was estimated at (328.75 mg EAG / g) and amount of flavonoids in plant phenol extract is estimated at (328,75mg ER/g) for the region of Hassani was better than Hamraya region wich was estimated at (220,312 mg ER / g). It can be argued that the high rate of flavonoids in the phenolic extract of *Citrullus Colocythis* of region of hassani is responsible for this activity. The result of the study of the effectiveness of anti-bacteria *Escherichia coli* where we recorded a very effective inhibitory ability at the extract for the region Hassani estimated at 18 mm at the largest concentration. **Conclusion:** In light of the encouraging results obtained with plant extracts, the qualifications of the plant can be estimated as a therapeutic plant containing inhibitors for the growth of some harmful bacteria. Its use as a natural antibiotic due to the rich presence of secondary metabolites.

Key words: *Citrullus Colocythis*, polyphenols, antibacterial

Introduction

The *Citrullus Colocythis* is among the natural products of medicinal plants, which was used in ancient medicine and treatment because of its many benefits used in the treatment was used in the treatment of many diseases.In this work, we conducted a comparative study of the: *Citrullus Colocythis* for two areas of Oued Souf (Hamraya and Hassani).This study was conducted to study the natural products found in the *Citrullus Colocythis* through the extraction of these product naturals and the study of antibacterial activity (Akobundu et al., 1982; Rajamanickam et al., 2010; Abdel-Hassan et al., 2000).

Pharmaceutical companies use many medicinal plants in the manufacture of treatments and drugs that contain in their composition useful substances to combat many diseases, because they are characterized by the absence of harmful chemicals with bad side effects, and from this standpoint, it is good to return to the use of these herbal medicinal plants in our lives Every day, which represents a new trend in the course of alternative medicine therapy, so we must know the best benefits of these medicinal plants in treating diseases, namely *Citrullus Colocythis* (Chunekar and Pandey, 1998; Meena and Patni, 2008; Gurudeeban et al., 2010).

Chemicals and reagents

Methanol, chloroform, n-butanol, petroleum ether, and ethyl acetate were purchased from VWR Merk (France), folin-ciocalteu reagent, Gallic acid, querctin, were procured from Sigma–Aldrich Inc (Paris, France). The reagents for the microbial activity were Nutrient agar and sabouraud dextrose agar.

Plant material

The fruits of the *Citrullus Colocythis* plant were collected were collected in Mars 2019 from a village in Hassani and Hamraya of El Oued state, Algeria and were identified by a botanist (Heliss Youssef) at the herbarium in the Department of Chemistry , the University of El-Oued, Algeria. The fruit was washed using water, dried at room temperature, and grounded into powder and then stored at room temperature until use.

Scientific classification

Citrullus Colocythis is a species of *C. colocynthis* . These plants are *Citrullus* is one of the perennial herbaceous plants that grow crawling on Earth. It follows cucurbitales, its green spherical fruits, striped in yellow to white, smooth and spongy pulp. Inside, there are harsh seeds known as *Citrullus colocynthis* seeds show in **picture 1** (Tannin-Spitz et al., 2007).

Kingdom: Plantae	Clade: Tracheophytes
Order: Cucurbitales	Family: Cucurbitaceae
Genus: Citrullus	Species: C. colocynthis

Binomial name:

Citrullus colocynthis (Sawaya et al., 1983).

Picture 1: The fruits of the plant *Citrullus colocynthis*

Preparation of extracts

100g of the *Citrullus colocynthis* material were macerated three times in a hydroalcoholic mixture (ethanol /water; 70/30; V/V) with renewed solvent every time for 24 hours. After filtration, the extract underwent successive liquid-liquid extractions using increasing polarity solvents starting with petroleum ether then ethyl acetate and finally methanol (Tannin-Spitz et al., 2007; Sawaya et al., 1983).

HPLC Analysis

The Phenolic compounds have been separated and identified by liquid chromatography system high-performance reverse phase mark (SHIMADZU, Japan). equipped with a UV diode array detector (DAD) and a chromatographic column filled with a grafted silica gel, octadecyl type RP-HPLC- C18 (25cm x 46 mm). The detector (DAD) was adjusted to a scan from 200 to 400 nm, whereby the column temperature was maintained at 25 °C. The volume injected was 20 µL and the mobile phase used was made up of two solvents A and B: Solvent A (acetonitrile), Solvent B (acetic acid 0.2%). The separation method adopted was the gradient elution with a speed set at 1 mL/min.

Identification of phenols and flavonoids were performed by comparing the retention times with authentic compounds injected in the same chromatographic conditions (Hamza et al., 2016).

Determination of total phenolic content (TPC)

The total phenolics content of each extract was determined using the Folin–Ciocalteu's reagent (FCR). Briefly, a dilute solution of Gallic acid in methanol (0.3-0.03 mg/mL) was mixed with 0.5 mL of folin-ciocalteu reagent, followed by 0.8 mL of Na2CO3 (7.5 %). The reaction mixture was incubated for 30 min in a dark room. The absorbance of the mixture obtained is directly measured by UV-Visible spectrophotometer at 765 nm. The concentration of total phenolics in the extracts was expressed as mg of Gallic acid equivalent (GAE) per g of dry weight. The obtained correlation coefficient of the calibration curve was R^2 = 0. 999. All results presented are means (±SEM) and were analyzed in three replications (Silva et al., 2012; Kumaran, 2006; Khoudali et al., 2014).

Total flavonoids content (TFC)

This method is based on the oxidation of the flavonoids by sodium nitrite solutions (NaNO2, 5%) and aluminum chloride ($AlCl_3$, 10%) leading to the formation of a brownish complex having a maximum absorbance of 510 nm.

The observed OD was compared to that obtained by a known concentration of Rutin used as standard. In each of the test tubes, we add 500 µL from Quercetin solutions in methanol (between 0.3-0.03 mg/mL) at different concentrations. Then, we put successively 75 µL of a solution of NaNO2 (5%). After 5 minutes we add 125 µL of $AlCl_3$ (10%) and after 6 minutes we add 500 µL of NaOH (1N) and 500 µL of distilled water.

The reaction mixture was incubated for 30 min in a dark room. A calibration curve is prepared at different concentrations with standard solutions of Rutin. The absorbance of the mixture obtained is directly measured by a UV-visible spectrophotometer at 420 nm and the results are expressed in mg Rutin equivalent /g of dry matter (QE /g DM). The data were analyzed in three separate experiments. The obtained correlation coefficient of the calibration curve was R^2 = 0. 998 (Dewanto et al., 2002; Dziri et al., 2012; Liu et al., 2008; Silva et al., 2012; Chang et al., 2002).

Statistical analysis

Data were analyzed using statistical tests whereby the obtained results were presented in mean values, and standard deviations (SD). Since all measurements were carried out in three experiments, therefore, all the analyses in the present study were analyzed three times determinations. Statistical calculations were carried out by MS Excel 2007 software, correlations were obtained by Pearson correlation coefficient using bivariate correlations test. P value was set at 0.05. Therefore, the obtained value less P value (0.05) was regarded as a statistically significant and P values < 0.01 was regarded extremely statistically significant.

Antimicrobial activity assays

Different bacterial strains are treated in a diffusion method. We obtain Ethanol extract solutions with three concentrations of you for each extract and then saturate the disks in a petri dish. The results of the antibacterial activity of phenolic extracts of studied are against a set (*Mirococcus luteus, Vibrio vulnificus, Staphylococcus, Escherichia coli*). After incubation for 24 hours, we measure the diameters of the inhibitors of the extracts, the best against the bacteria *Escherichia coli.*

Incubation conditions

Nutrient agar was used culture medium for bacteria which was incubated for 24 h at the temperature of 37 °C and yeasts were cultured in sabouraud dextrose agar (SDA; 4% dextrose, 2% neopeptone and 1,7% agar) for 24-48 hours at the temperature of

30°C (Efstratiou et al., 2012; Rodzali and Mydin, 2017).

All strains were obtained from the Laboratory of Microbiology ELMAJD EL OUED 39000, Algeria.

Disc diffusion assay

Ethanol extracts of *Citrullus Colocythis* were dissolved in methanol-water 50% for a final concentration (10, 1, 0.1 mg/ml) and filter-sterilized through a 0.45 membrane filter. The antimicrobial activity was estimated by the method of disc diffusion, 100 µl of suspension for each microorganism 108 colony-forming units (CFU)/ml containing 20 ml of nutrient agar for bacteria, after was placed in the Petri sterilized filter paper disc (7 mm in diameter).

Disks were saturated with different concentrations of phenolic extracts inside Petri dish with reference disk saturated with methanol 50%. we let it in an inverted position in the incubator under the temperature 37°C for 24 hours.

The diameter of the inhibition zone around each disc was measured for three replicates

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Results

Extract yield

The Ethanol is a solvent extract the significant amount of phenolic compounds and recently used in several studies. It is considered as the best solvent substances compared with the other solvents. The results of extract yield for each *Citrullus Colocythis* are mentioned in **Table 1.**

Table 1. Mass yield of leaves obtained by phenolic extract for *Citrullus Colocythis*

Phenolic extract palm pollen	**Dry weight extract g/100 g of plants powder**
phenolic extract for *Citrullus Colocythis* in the region Hamraya	5.2 ± 0.07
phenolic extract for *Citrullus Colocythis* in the region Hassani	6.68 ± 0.17

Note : Results are expressed as the mean and ±standard deviation of three independent experiments.

Identification by HPLC

The identification of compounds phenolic extracts the majority of phenolic extract for *Citrullus Colocythis* in the region Hamraya and Hassani, HPLC were carried out on the basis of the comparison of their retention times with those obtained for the same standard compounds.

As show in **Fig. 1** and **Table 2**, polyphenols and Flavonoids rates are determined in plant extracts according to the calibration curve (peak areas as a function of the concentration of the standards).

Meaning of symbols:
PPH_{ph}: phenolic extract for *Citrullus Colocythis* in Hamraya region.
PPA_{ph}: phenolic extract for *Citrullus Colocythis* in Hassani region.

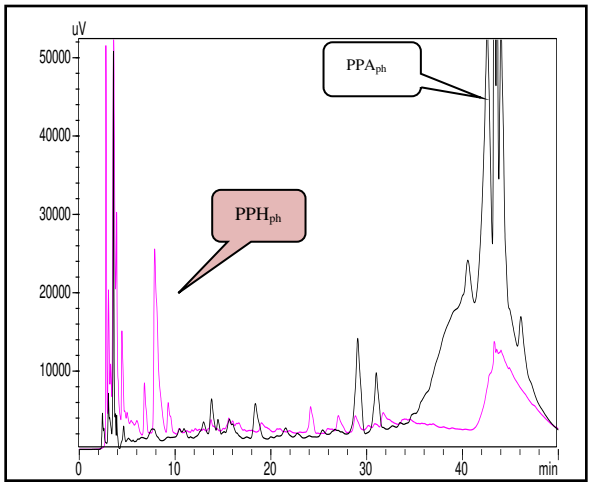

Figure 1: Chromatographic profile of the extract Recorded in UV at 254 nm.

Table 2. Quantification of phenolic and flavonoids compounds identified in a Ethanoic extract from *Citrullus Colocythis*

Phenolic compounds	$t_{r(R)}$ (min)	PPH_{ph}(mg/g)	PPA_{ph} (mg/g)
Ascrobic acid	4.21	0.078	0.261
Gallic acid	5.23	0.189	0.18
Chlorogenic acid	13.62	0.739	1.085
Quercetin	20.37	2.671	0.056
Caffeic acid	16.3	1.199	0.289
Vanillin	21.46	0.606	0.32
p-Coumaric acid	23.95	0.864	0.027
Rutin	28.22	1.062	2.564

Total phenolic and flavonoids

The results of the quantitative analyses of polyphenols and flavonoids in the extracts of PPH_{ph} and PPA_{ph} , are reported in **Table 3**.

Table 3. Total Phenolic and flavonoids content of PPH_{ph} and PPA_{ph}

Ethanoic extract from *Citrullus Colocythis*	**TPC (mg GAE/g)**	**TFC (mg ER/g)**
PPH_{ph}	220.312± 0.1	12.5± 0.03
PPA_{ph}	328.75± 0.2	15.25± 0.07

Antimicrobial activity

After incubation for 24 hours, we measure the diameters of the inhibitors of the extracts and antibacterial activity of extracts and antibiotics on the three bacterial strains, resulting from different concentrations of extracts are shown in **Table 4.**

Table 4. Diameters bacteria's inhibition resulting from different concentrations of antibiotics and extracts

Microorganisms Bacteria	Diameter of zone inhibition(mm)	
	PPH_{ph}	**PPA_{ph}**
Vibrio vulnificus	ND	12± 0.02
Mirococcus luteus	ND	ND
Escherichia coli	17± 0.05	18± 0.07
Staphylococcus	14± 0.03	16± 0.05

Note : ND : no detected

Discussion

Extract yield

From **Table 1**, which shows the extraction yield (g/100 g dry weight), the mass yield obtained phenolic extract for *Citrullus Colocythis* in the Hamraya region is 5.2 % and phenolic extract for *Citrullus Colocythis* in the Hassani region is 6.68 %. Where we can suggest that the reason for this difference in yield percentages is due to the difference in soil quality from one region to another one, which affected the yield of the product.

HPLC analysis

From Fig.1 and Table2, and by comparing the chromatographic of sample PPH_{ph} and chromatographic of sample PPA_{ph} , we conclude that the extracts contain the eight reference phenolic compounds previously identified, but their quantities differ from one extract to another, either through the area of the peaks or through recorded quantities such as Gallic acid, Caffeic acid, Quercetin, vanillin, and *p*-coumaric acid ,were greater in Sample PPH_{ph}, while ascorbic acid, chlorogenic acid and Rutine in Sample PPA_{ph} are larger than Sample PPH_{ph}

This diversity can be attributed to many biological factors, including genetic and agricultural differences, as well as other environmental factors, such as stages of ripeness, salinity, temperature, water pressure and conditions of light intensity. HPLC analysis provides insight into phenolic compounds in *Citrullus Colocythis* and change of phenol and flavonoids.

The results presented in this study are similar to previous studies (Daoud et al., 2019), and are almost similar in terms of the type of phenolic compounds that make up them, and the difference is only relatively in quantities.

This result can be explained by the physiological role of every compound during the different stages of growth, for gallic acid was been helped to adaptation the plans with the climatic conditions. (Weidenhamer et al., 2013) The Caffeic acid has been accelerated to aging of plants The vanillin does accelerate the maturity of fruits by modifying the taste and flavor of the fruit (Schnablová et al., 2006) and it has the role of anti-abiotic stress. As for ρ-Coumaric acid has a role in the reduction of the vegetative growth of the plants and it is a good stimulated for antibacterial activity (Schnablová et al., 2006; Dakshini and Foy, 1999).

Total phenolic and flavonoids

In **Table 3**, The technique methods of estimation of total phenolic. The result regarding the first PPHph. The total phenolic content of different extraction techniques ranges from 220.312mg EAG /g, the content of flavonoids in Rutin equivalent varies from 12.5 mg ER/g. Regarding to the second PPAph, with same method the results range from 328.75 mg EAG /g, the content of flavonoids in Rutin equivalent varies from 15.25 mg ER/g

From the results of PPAph extracts generally exhibited higher polyphenolic contents than the PPHph extracts, this behavior could be attributed to the geographical variations, and climatic changes. Overall,the findings indicated that both PPAph and PPHph are rich in phenolic and flavonoid contents, which could be the major contributor to their anti- microbial properties. These result ssuggest that both varieties of *Citrullus Colocythis* offer promising sources of beneficial bioactive compounds for human health and nutrition.

These results were much better than some researchers have found in this field (Kumar et al., 2008) this difference is due to the harsh desert nature in which it grows in the well-known Oued Souf region, which is among the hottest regions in the world.

Overall, the results indicate that PPAph can be considered a promising source of new natural antioxidants and antimicrobial agents for use in various products, pharmaceuticals, and medicines.

Antimicrobial activities

In **Table 4** we observe the best inhibition diameter against the bacteria *Escherichia coli* the highest inhibition value was recorded by the phenolic extract of the Hassani region of 18 mm.

It can be said that *E* .coli has moderate sensitivity to the highest concentrations of extracts.

The best inhibition diameter against the bacteria *Staphylococcus* the highest inhibition value was recorded by the phenolic extract of the Hassani region of 16 mm.

It can be said that *E.coli* has moderate sensitivity to the highest concentrations of extracts.

Inhibition diameters were not seen in all concentrations and in all samples against *Mirococcus luteus*. This is due to the fact that *Mirococcus luteus* bacteria have a natural resistance.

The phenolic extract of the Hamrya region concentrations did not

show any inhibition diameters, whereas the phenolic extract of the Hassani region inhibition diameter was 12 mm at highest concentration and therefore *Vibrio vulnificus* bacteria were medium sensitive to highest concentration.

From the study of antibacterial activity of extracts and antibiotics on the four bacterial strains showed that the ability of the extracts to inhibit E.Coli bacteria was better, the highest inhibition value was recorded by the 18 mm diameter phenolic extract in the Hassani region.

Conclusion

The phenolic extract for the region Hassani was better than Hamraya region where it was estimated at (328.75 mg EAG / g).

We also identified some phenolic compounds for extracts through qualitative analysis using a high-performance liquid chromatography device (HPLC), where we recorded the presence of all reference phenolic compounds (08compounds) which are: ascorbic acid, gallic acid, chlorogenic acid, caffeic acid, Quercetin, vanillin, p-coumaric acid, and Rutin.

The result of the study of the effectiveness of anti-bacteria *E.coli* where we recorded a very effective inhibitory ability at the extract of extract for the Hassani region estimated at 18 mm at the largest concentration.

Ultimately we hope to futur study the quantitative and qualitative analysis of the active compounds by identifying the chemical formulas of the plant compounds responsible for this activity.

Data Availability

All data generated or analyzed during this study are included in this published article

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Authors' contributions

Tamma Noureddine directed the study process and evaluated the research results and revised manuscript. Rebiai AbdelKarim analyzed Data, and , Naima BENCHIKHA led the interpretation of the data in this article.

All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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