Int.J.Curr.Microbiol.App.Sci (2024) *13*(11): 262-280

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 13 Number 11 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1311.029

Phytochemical Screening, Anti-bacterial and Anti-fungi Activities of Leafs, Stems and Roots of *C. parviflorus* Lam and *C. salviifolius* L Plants

Hamad. M. Adress. Hasan[1*], Najat. Ben. Areos[2] and Naser. M. Emaza[3]

[1]Department of Chemistry, Faculty of Science, Omar Al –Mukhtar University, Libya
[2]Department of Chemistry, Faculty of Arts & Science, Al Mergab University, Libya
[3]Department of Chemistry, Faculty of Science, AlMergab University, Libya

**Corresponding author*

Keywords

Phytochemical screening, biological, *C. parviflorus* Lam

Article Info

Received:
10 September 2024
Accepted:
26 October 2024
Available Online:
10 November 2024

A B S T R A C T

This study was carried out on leafs and steams and roots of *C. parviflorus Lam* and *C. salviifolius* plants, which growing at Al–Gabal Al–Akhder region, Libya. The phytochemical screening, anti-oxidant and total phenol contents were determined. Two different solvents were used in this study (Aqueous and Acoholl). The anti-microbial investigation was carried out on the plant extracts against different types of pathogenic bacteria including (*Bacilli*, *Streptococcus*, *E. coli* and *Pseudomonas*), beside three types of Fungi including (*Aspergillus niger*, *Rhizopus* and *Fusarium*). The results recorded that, there are variations in phytochemical screening, where the detected compounds of: carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, and anthraquinone, showed variations in both extracts of the selected plant samples. Also the extracts were used for antibacterial and antifungal application studies, the results showed different effects on the selected microbial and fungi species which selected in this study, where some of extracts gave inhibition zones compared with those which no gave the effect on the studied bacteria and fungi species.

Introduction

Natural products such as plants extract open a new horizon for the discovery of new therapeutic agents (Cosa *et al.,* 2006). Medicinal plants are right in considered of great importance due to their properties as an expansive source of helpful phytochemicals which will lead to the development of novel drugs. Most of the phytochemical from plants sources such as phenols and flavonoids have been detailed to have positive effect on health and cancer avoidance (Venugopal and Liu, 2012). Medicinal plants have been utilized since old times to treat against illnesses (Bouyahya *et al.,* 2017). Through the chemical components of the plants, their medicinal properties are determined (Ezeonu and Ejikeme, 2016).

The World Health Organization (WHO) has documenting medicinal plants used indigenous people from different parts of world. The use of plant derivatives as medicinal treatments gained popularity in the late 1990. The use of

traditional medicine and medicinal plants in most developing countries as a standard basis for maintaining good health is widely observed and about 80% of the world's population depends on herbal medicine (UNESCO, 1996).

Plants generally produce several secondary metabolites like phenols, flavonoids, quinones, tannins, alkaloids, saponins, and sterols which are important sources of biocides and many other pharmaceutical drugs (Naili, 2010).

Medicinal plants are important in pharmacological research and drug development (Li and Vederas, 2009). About 7.000 medicinal compounds used in the western pharmacopoeia are derived from plants (Muhaisen *et al.,* 2016). Plants have been a rich source of medicines because they produce wide types of bioactive molecules, most of which probably evolved as chemical defense against predation or infection and antioxidant compounds.

Many plants possess antimicrobial activities and different activities are used for the treatment of different diseases (Arora and Kaur, 1999). The Arab world began to excavate its own older works, and the Renaissance years saw a renaissance in ancient medicine, built largely on medicinal plants. Plants have an almost unlimited ability to synthesize aromatic compounds, the bulk of which are phenols.

The use of such alternative medicines has become ever more common in the advanced world. Libya has an enormous wealth of medicinal plants, In libya many thousands of medicine plants are naturally growing at different areas, especially in the Al-Jabal Al-Akhtar region and these plants are used for their medicinal qualities in folk medicine (Iwu *et al.,* 1999). Medicinal plants have been used in folk medicine in libya rural areas ay relatively cheaper expenses than modern medicine, they have been widely used as diuretics, topical anti inflammants, and haemostatic (Burt, 2004). In this study the *Cistus* plant which growing at Al-Gabal Al-Khder (Libya) was selected

The Mediterranean locale is known to be the normal territory of the genus cistus (Skoric *et al.,* 2012). These plants are able to grow in difficult climatic and soil dray or rocky conditions (Aronne and Micco, 2001). The straight branches can run from (50 to 100 cm) in high. On the branches, leafs grow develop entangled and clear (Zeyaullah *et al.,* 2009). The main *Cistus*species found in the Mediterranean basin namely *C. albidus, C. creticus, C. crispus, C. parviflorus, C. monspeliensis, C. populifolius, C. salviifolius, C. ladanifer, C. laurifolius,* and *C. clusii* (Papaefthimiou *et al.,* 2014). Most of the species of this family that have a sweet and fragrant aroma are highly prized in the perfume industry (El Euch *et al.,* 2015).

Chemical studies, conducted on different species of the genus *Cistus*, revealed that their components consist of terpenes, flavonoids, phenolic acids, resacetophenoneglucoside, and bornyl esters (Fang *et al.,* 2018). Essential oils, resin, gum and lipids (from the seeds) (Guvenc *et al.,* 2008). The considers fundamental oil composition of *Cistus*species uncovered the presence oxygenated monoterpenes, sesquiterpenes, aromatics, oxygenated sesquiterpenes and traces of carbonyl compounds (Mastino *et al.,* 2017). Leafs of *Cistus* species are convered with organs emitting resin and basic oil comprising basically of terpenoids. The leafs and stems of these lasting bushes have glandular tri chomes emitting a gum basically amid the summer months (Skoric *et al.,* 2012). Two types of *Cistus* plant were selected in this study including: *Cistus salviiflius* and *Cistus parviiflous*.

Phytochemistry is study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants (Singh, 2015). Phytochemical screening of aqueous and alcoholic extracts of leafs, steams and roots of (*Cistus parviflorus lam* and *Cistus salvifolius* L) plants which growing at Al-Gabal AL-Akhder region in Libya. Measuring the anti-oxidant activity of the studied plant samples.

Determination of the total phenolic contents of the selected plants. Evaluation the antimicrobial activity of aqueous and ethanol extracts from leafs, stems and roots of *Cistus parviflorus Lam* and *Cistus salviifolius L* against a variety of microorganisms (Bacteria and Fungi).

Experimental Part

Sampling

Due to the importance of many plants which used at AL-Gabal AL-Khder region (Libya), this study was designed to select two plants (*C. parviflorus* and *C. Salviifolus*). The samples were collected from Al-Gabel Al –Kadar

region during spring season of (2022) year. Plants Taxonomy: The collected samples were identified in *Seliphium* herbarium, Botany Department, Faculty of Science, Omar Al- Mukhtar University.

Samples preparation

The leafs, stems and roots of the studied plants were separated and washing several times with distilling water. The samples then dried in dark and dry place. Then the samples were grinded by mortar and stored in polyethylene bottles until analysis (Hasan *et al.,* 2011).

Phytochemical screening

All the phytochemical screening tests were carried out according to standard methods. The methods are described as following:

Test for sterols and/or triterpines

The sterols and/or triterpines compounds were detected by used Libermann-Burchad's test: where, (one ml) of the chloroform extract of each sample with (0.3 ml) of acetic anhydride were mixed, then few drops of concentrated sulphuric acid were added along the side of the dry test tube. Reddish-violet colour is produced at junction of the two layers and chloroform solution acquire green colour in case of presence of sterols and/or triterpenes (El Hifnawy *et al.,* 1992).

Test for flavonoids

The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with 1% hydrochloric acid; each extract was subjected to the following test, 10 ml of each extract is rendered alkaline where faint yellow colour is produced in case of presence of flavonoids (Balbaa *et al.,* 1981).

Test for alkaloids

The extracts of the tested herbal plants were further extracted with 20ml of dilute hydrochloric acid, cooled and rendered alkaline with dilute ammonium hydroxide solution, then extracted with chloroform.

The chloroform extract is subjected to the following test: Dragendorff: The preparation of the reagent: Solution (a): About of (0.85 g) of basic bismuth nitrate was dissolved in mixture of 10 ml acetic acid and 40 ml distilling water Solution (b): about (8 g) of potassium iodide was dissolved in (20) ml water. Stock solution: Equal volumes of solutions (a) and (b) are mixed. Few drops of chloroform extract was applied to filter paper, allowed to dry and sprayed with the reagent. Orange colour is observed in cases of the presence of alkaloids.

Test for tannin

The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with ethanol 50%, filter, and the hydro-alcoholic clear solution is subjected to the following test:

Ferric chloride test

One ml of the reagent (1% $FeCl_3$) was added to the hydro-alcoholic solution. Blue colour develops in cases of the presence of pyrogallol tannins (Clauss, 1961 and Egyptian Pharmacopoia, 1984).

Carbohydrates and /or glycosides

Two ml of the extract is mixed with 0.2 ml ethanolicα-naphthol (20 %) and 2ml of concentrated sulphuric acid was added on the side of the dry test tube.

Violet ring is observed at the junction of the two layers cases of the presence of carbohydrates and/or glycosides (Clauss, 1961).

Tests for cardiac glycosides

Keller-Killiani test

One ml of each extract of the tested herbal preparations was dissolved in glacial acetic acid containing traces of ferric chloride; concentrated sulphuric acid containing the same amount of ferric chloride is placed at the bottom of the test tube with a pipette where intense blue colour at the surface between the reagents develop for 2-5 minutes, spreading gradually into acetic acid layer, in cases of the presence of deoxy-sugars.

Anthraquinones

One ml of each extract of the successive extracts of the tested herbal preparations is hydrolyzed with alcoholic potassium hydroxide, the acidified and continues as Bornträger's test.

Rose-Red develops in the aqueous layer in cases of the presence of anthraquinones (Egyptian Pharmacopoia, 1984).

Saponins

Five ml of tape water is added to (1 ml) of each extract, then shaken vigorously for five minutes, froth develop having (1 cm) high and persists for (15 minutes) indicates the presence of saponin (Clauss, 1961). In this study the phytochemical screening, antioxidant activity, total phenols analysis of the studied samples were expressed as the following Codes:

Chemical studies

Determination of antioxidant capacity by Prussian Blue method

One gram of the sample powder was defatted with petroleum ether. The defatted powder was then extracted sequentially by stirring with 10 ml methanol twice, then with 10 ml 1% hydrochloric acid: methanol(v/v). The three combined extracts were evaporated under vacuum and the residue was dissolved in 10 ml methanol.

Half ml of the solution was diluted with 3 ml distilled water, 3 ml (0.008 M) of $K_3Fe(CN)_6$ Was added, 3 ml 0.1M Hcl, and 1 ml 1% $FeCl_3$. The blue color is allowed to develop for 5 min and the absorbance is measured at 720 nm against the blank.

Determination of total phenols by FolinCiacalteu Method

This method was carried out to determine phenolic compounds the aqueous and Ethanol extracts, where 10 ml was added to 3ml of distilled water with FolinCiacalteu reagent. According to the method of Slinkard and Singleton that using gallic acid as a standard.

Samples (leafs and seeds of barley plant) were introduced into test curettes', and then 0.5mL of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (20%) were added. The absorbance of all samples was measured at 650 nm using UV–Vis spectrophotometer after incubating at (1 min) and cooled for (15 min). Results were expressed as milligrams of gallic acid equivalent per gram of fresh weight.

Antimicrobial activity

Preparation of the extracts

Fresh of the studied plants were washed two times tap water and subjected to shade drying at room temperature the dried plant material was powdered using a mechanical grinder (Alshammary and Ibrahim, 2014).

Solvents extraction

The powdered of the plants were extracted with different solvents, where (10 grams) of each plant powders were added to (150 ml) of aqueous and non-aqueous solvent of (Ethanol). Crude extract was evaporated at 65 ^{0}C for aqueous extracts and at 45 ^{0}C for alcoholic extracts, with the rotary evaporator, then the extracts were collected and stored at 4^{0}C until further uses (El-Hifnaway *et al.,* 1992).

Microorganisms

The extracts were individually tested against pathogenic bacteria, the following bacteria were tested:

Bacterial strains

Gram positive bacteria

Two species of Gram positive (*Streptococcus* and *Bacillus cereus)* were selected in this study.

Gram negative bacteria

The species of Gram negative (*Escherichia coli and Pseudomonas aeruginosa)* were selected in this study.

Fungi strains

The fungi species which selected in this study including: (*Fusarium*, *Aspirgillus niger* and *Rhizopus*). All microorganisms species were obtained from Department of microbiology of Faculty Veterinary Medicine Omar Al- Mukhtar University.

Software programs

Excel program was used to draw the figures and calculate the statically measurements of average and standard Deviation values.

Results and Discussion

The results of phytochemical screening of the studied samples

The discussion of phytochemical screening is depend up on the color test (qualitative tests). Each solvent extracts of the studied plants were screened for the following constituents: carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, and anthraquinone. The obtained results were recorded in the Tables (1 and 2), the results revealed presence of carbohydrates and/or glycosides, sterols and/or triterpenes, tannins, saponin, and flavonoids alkaloids, anthraquinone and cardiac glycosides were recorded in all studied plants species with presence different contents. The sterols and/or triterpenes was detected in all extracts of the studied plants of *C. parviflorus Lam* and *C. salviifolius* in both alcoholic and aqueous extracts. High contents were observed in leafs extractions comparing with steams and roots of the both plants. On the other side low contents were observed in steams. Generally alcoholic extracts showed high contents of sterodies comparing with aqueous extracts. For the Flavonoides compounds the results showed that, absent of Flavonoids in the extracts of (aqueous roots extracts, leafs of aqueous extracts of *C. salviifolius L* plant and steams of *C. parviflorus Lam* plant. The contents of Flvonoides showed variations in their contents of the studied extracts, relative high contents were observed in alcoholic extracts of steams of *C. salviifolius L* comparing with the other extracts. Alkaloids was detected in all the studied extracts, generally there is relative increasing of their contents in aqueous extracts comparing with alcoholic extracts. The results showed low contents in steams of alcoholic extracts of the both studied plants, roots of the two studied plants exhibit high contents of Alkaloids. The tannins compounds were detected in all extracts of *C. salviifolius L* plant, high contents of tannies were observed in leafs of the studied plants, but the tannies not detected in roots of *C. parviflorus Lam* plant of both alcoholic and aqueous extracts. Generally tansies were showed higher contents in most of the studied plants comparing with other constituents. The carbohydrates and /or glycosides were detected most the extracts of the both studied plants except for alcoholic extract of leafs of *C. salviifolius L* plant and alcoholic extracts of leafs extract and aqueous extract of roots of *C. parviflorus Lam* plant. The results gave indication that the *C. salviifolius L* plant containing high contents of carbohydrate and /or Glycosides comparing with *C. parviflorus Lam* plant. For the cardiac glycoside compounds the results showed presence of them in all *C. salviifolius L* extracts of both aqueous and alcoholic of leafs and steams and roots, whereas the cardiac glycoside compounds were not detected in extracts of (Alcoholic leafs, aqueous steams of *C. parviflorus Lam* plant). The results also indicated that there are high contents of cardiac glycoside compounds in (leafs, steams and roots of *C. parviflorus Lam* plant). The Anthraquinones compound groups were detected in all extracts of *C. parviflorus Lam* plant (except in leafs), on the other side the Anthraquinon compounds not detected in the extracts of *C. salviifolius L* plant except in (aqueous extracts of steams and roots), there is relative increase in anthraquinone contents in alcoholic extract of roots of *C. parviflorus Lam*. The results of saponins showed presences of these compounds in all the studied extracts except in (alcoholic extracts of leafs and steams) *C. salviifolius L* plant, also the results exhibited that the roots containing high contents of saponinis especially in alcoholic extracts for the two studied plants. Relative increase in saponins contents were detected in aqueous leafs extracts of the studied plants. While the aqueous steam extracts showed low contents of saponins.

Antioxidant Capacity

The obtained results of anti –oxidant capacity are mentioned in Table (3) and shown in the Figure of (1). The antioxidant capacity of the studied plants showed that they contain high levels of antioxidants in the leafs, steams and roots of *C. parviflorus* and *C. salviifolius* plants. were the highest concentration recorded in leafs of *C. salviifolius* (69.97 ppm) followed by steams of *C. salviifolius*, leafs of *C. parviflorus*, roots of *C. parviflorus*, steams of *C. parviflorus* and roots of *C. salviifolius* (56.731, 53.490, 52.564, 50.898 and 50.620 ppm), respectively. The results in this study showed high levels of antioxidants capacity of leafs, steams and roots of studied plants.

Total phenols

The concentrations of total phenols contents of the studied plant extracts of *C. parviflorus* and *C. salviifolius* was determined using folin-ciocalteu method, the obtained results were illustrated in the Tables of (4 & 5) and represented in the Figures of (2 & 3). The results showed that this species is very rich in total phenols. Total phenols content was found e in ethanol extracts of *C. parviflorus* leafs (363.32 ppm), while total phenols

content was found in aqueous extracts of *C. parviflorus* leafs (120.397 ppm). Total phenols content were found to be in ethanol extracts of *C. parviflorus* steams (221.77 ppm) while total phenols content was found in aqueous extracts of *C. parviflorus* steams (159.79 ppm). Total phenols content was found to be in ethanol extracts of *C. parviflorus* roots (275.99 ppm), while the high total phenols content was found in aqueous extracts of *C. parviflorus* roots (304.37 ppm). Total phenols content were found in ethanol extracts of *C. salviifolius* leafs (619.39 ppm) while total phenols content was found in aqueous extracts of *C. salviifolius* leafs (180.27 ppm). Total phenols content was found to be in ethanol extracts of *C. salviifolius* steams (519.96 ppm) while total phenols content was found to be in aqueous extract of *C. salviifolius* steams (338.10 ppm). Total phenols contents were found to be in ethanol extracts of *C. salviifolius* roots (354.30 ppm) while total phenols content was found to be in aqueous extracts of *C. salviifolius* roots (197.14 ppm). Ethanol extracts of *C. parviflorus* and *C. salviifolius* contained higher amounts of total phenols than aqueous extracts and ethanol extracts of *C. salviifolius* contained the highest amount of total phenols, followed by ethanol extracts of *C. parviflorus*. The folin-ciocalteu method is a rapid and widely used assay, to detect the total phenolic content but it is known that different phenolic compounds have different responses in the folin-ciocalteu method (Kahkonen *et al.,* 1999). Phenolics and carotenoids form the basis of antioxidant activity (Mullen *et al.,* 2007). phenolic and flavonoid compounds have been reported to be responsible for the antioxidant activities of medicinal plants and other botanical material (Mohamed *et al.,* 2010). In this study the high phenol contents of both plants *C. parviflorus* and *C. salviifolius* indicate an important health promoting activity as antioxidant, anticancer, antiflammatory and biological effects and this is in agreement with several previous studies.

Anti-microbial activity Results

Anti-Bacterial activity studies

The anti microbial activity studies were carried out on the aqueous and alcoholic extracts for leafs, steams and roots of the studied plants against some species of bacteria and fungi. The samples used for anti –microbial investigations of this study were taken numbers of (1-12) as shown in Table (5). The results of antimicrobial tests are shown in Tables (), and Figures (), and described as following:

Antibacterial activity

Gram negative bacteria

Escherichia coli

Tables (6 & 7) and Figures (4-6) showed the effect of different extracts of studied plant against *Escherichia coli*. The results showed that the inhibition zone of (5 and 7 µm) were recorded for of aqueous extracts of steams and roots of *C. savifolius L* plant, respectively, whereas no anti- bacterial activities were recorded for leafs, steams and roots of aqueous extracts of *C. parviflourus Lam.*

On the other side all the alcoholic extracts of studied plants gave anti-bacterial activities, the high activity values of (10 µm) were recorded for the root extracts for the both plants of *C. parviflourus Lam* and *C. savifolius L.*

Pseudomonas aeruginosa

Tables (6 & 7) showed the effect of different extracts of the studied plants extract against *pseudomonas*. The results showed that the inhibition zone were recorded for all aqueous extracts, Figure (). High inhibition zone value of (20 µm) was recorded for the leafs aquouse extracts of the both plants of *C. parviflourus Lam* and *C. savifolius L.* Low inhabitation zone of (1µm) was recorded for the steams of *C. savifolius L* plants. On the other side no any *pseudomonas* activity was recorded for the roots extract of *C. savifolius L* and all alcoholic extracts of studied plants.

In this study the Antimicrobial activities of the studied samples was expressed as the following numbers:

Gram positive bacteria

Bacillus

By applying different extracts of studied plant extracts against *Bacillus cereus* the results showed that the inhibition zones and MIC of all extracts were recorded for the all aqueous and alcoholic extracts. (Tables 8 and 9) and represented in the Figures of (7 -9). But the results showed high effects (inhibition zone) of alcoholic extracts (3 -20 µm) on *Bacillus cereus* comparing with the aqueous extracts (1- 5 µm).

Table.1 Sample details

Sample Code	Sample Type
A	***C. parviflorus Leafs***
B	***C. parviflorus Steams***
C	***C. parviflorus Roots***
D	***C. saiviifolius Leafs***
E	***C. saiviifolius Steams***
F	***C. saiviifolius Roots***

Table.2 Phytochemical screening of leafs, steams and roots of *C. parviflorus Lam.*

Plant Test	***C. parviflorus Lam .***					
Chemical test	**Leafs**		**Steams**		**Roots**	
	Aq	Al	Aq	Al	Aq	Al
Steroids and/or Triterpenods	+++	+++	+	+	++	+++
Flavonoids	++	++	-	+	+	-
Alkaloids	++	+	++	+	++	+++
Tannines	++++	++++	++++	+	-	-
Carbhydrate and/or Glycosides	++	-	++	+	-	+
Cardiac Glycosides	++	-	-	+	+	+++
Anthraquinones	-	-	++	+	+	+++
Saponins	++	+	+	++	+	+++

(+)Present , (++) Moderate content , (+++) High content (Dark Color),
Al (Alcoholic) , Aq (Aqueous) and (-) Absent

Table.3 Phytochemical Screening of leafs, steams and roots of *C. salviifolius L.*

Plant Test	***C. salviifolius L***					
Chemical test	**Leafs**		**Steams**		**Roots**	
	Aq	Al	Aq	Al	Aq	Al
Steroids and/or Triterpenods	++	+++	+	++	++	++
Flavonoids	-	+	+	+++	-	-
Alkaloids	++	+	+	+	+++	+
Tannines	++++	++++	+++	++	++++	++++
Carbhydrate and/or Glycosides	+++	-	++	++	+++	+++
Cardiac Glycosides	+++	+++	+	+++	+++	+++
Anthraquinones	-	-	+	-	++	-
Saponins	+++	-	++	-	+	+++

(+)Present , (++) Mderate content , (+++) High content (Dark Color), Al (Alcoholic) , Aq (Aqueous) and (-) Absent.

Table.4 Antioxidant activity of leafs, steams and roots *C. parviflorus Lam and C. salviifolius L.*

Sample Code	Antioxidant (µg/g)
A	53.490
B	50.898
C	52.564
D	69.97
E	56.731
F	50.620

Table.5 Total phenols contents of leafs, steams and roots *C. parviflorus Lam* and *C. salviifolius L.*

Sample Code	Aq. Extract	Al. Extract
A	120.397	363.325
B	159.795	221.777
C	304.373	275.993
D	180.277	619.367
E	338.108	519.969
F	197.144	354.307
Average	**216.6823**	**392.4563**
± SD	**85.61305**	**150.1646**

Table.6 The sample numbers used in anti-microbial studies.

Sample No	Sample Type	Sample No	Sample Type
1	aqueous extract of leafs *C.Parviflorus* Lam	7	alcoholic extract of leafs *C.Parviflorus* Lam
2	aqueous extract of steams *C.Parviflorus* Lam	8	Alcoholic extract of steams *C.Parviflorus* Lam
3	Aqueous extract of roots *C.Parviflorus* Lam	9	Alcoholic extract of roots *C.Parviflorus* Lam
4	Aqueous extract of leafs *C.Salviifolius L*	10	Alcoholic extract of leafs *C.Salviifolius L*
5	Aqueous extract of steams *C.Salviifolius L*	11	Alcoholic extract of steams *C.Salviifolius L*
6	Aqueous extract of roots *C.Salviifolius L*	12	Alcoholic extract of roots *C.Salviifolius L*

Table.7 Antibacterial activities of crude aqueous extracts Leafs, steams and roots of *C. parviflorus Lam and C. salviifolius L.*

Sample No.	1	2	3	4	5	6
Escherichia coli	NA	NA	NA	NA	5µm	7µm
Psudomonas	20 µm	5 µm	5 µm	20 µm	1 µm	NA

Table.8 Antibacterial activities of crude alcoholic extracts Leafs, steams and roots of *C. parviflorus Lam and C. salviifolius L*

Sample No	7	8	9	10	11	12
Escherichia coli	2 µm	7 µm	10 µm	5 µm	5µm	10µm
Pseudomonas	NA	NA	NA	NA	NA	NA

Table.9 Antibacterial activities of crude aqueous extracts Leafs, steams and roots of *C. parviflorus Lam* and *C. salviifolius L.*

Sample No	1	2	3	4	5	6
Bacillus	1 µm	1 µm	1 µm	5 µm	1 µm	2 µm
Streptococcus	10 µm	5 µm	2 µm	10 µm	NA	15 µm

Table.10 Antibacterial activities of crude alcoholic extracts Leafs, steams and roots of *C. parviflorus Lam and C. salviifolius L.*

Sample No	7	8	9	10	11	12
Bacillus	6 µm	3 µm	20 µm	10 µm	5 µm	10 µm
Streptococcus	5 µm	10µm	10 µm	10 µm	5 µm	12 µm

Table.11 Antifungal activities of crude aqueous extracts Leafs, steams and roots of *C. parviflorus Lam* and *C. salviifoliusL* plants.

Sample No Fungi	1	2	3	4	5	6
Fusarium	5 µm	NA	NA	5 µm	NA	NA
Rhizopus	6 µm	4 µm	NA	2 µm	5 µm	5 µm
Aspergillus	NA	NA	NA	NA	NA	NA

Table.12 Antifungal activities of crude aqueous extracts Leafs, steams and roots of *C. parviflorus Lam* and *C. salviifolius L* plants.

Sample No	7	8	9	10	11	12
Fusarium	NA	NA	5 µm	5 µm	10 µm	11 µm
Rhizopus	NA	5 µm	15 µm	NA	5 µm	8 µm
Aspergillus	NA	NA	NA	NA	NA	NA

Figure.1 Antioxidant activity of leafs, steams and roots of *C. parviflorus Lam* and *C. salviifolius L.*

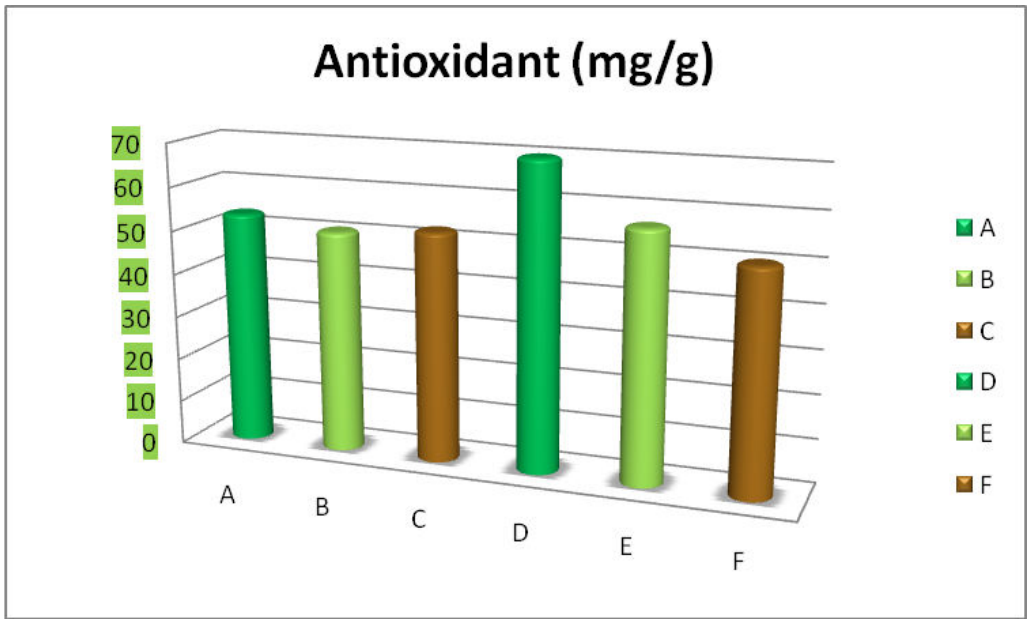

Figure.2 Total phenols content aqueous extract of leafs, steams and roots *C. parviflorus Lam and C. salviifolius L.*

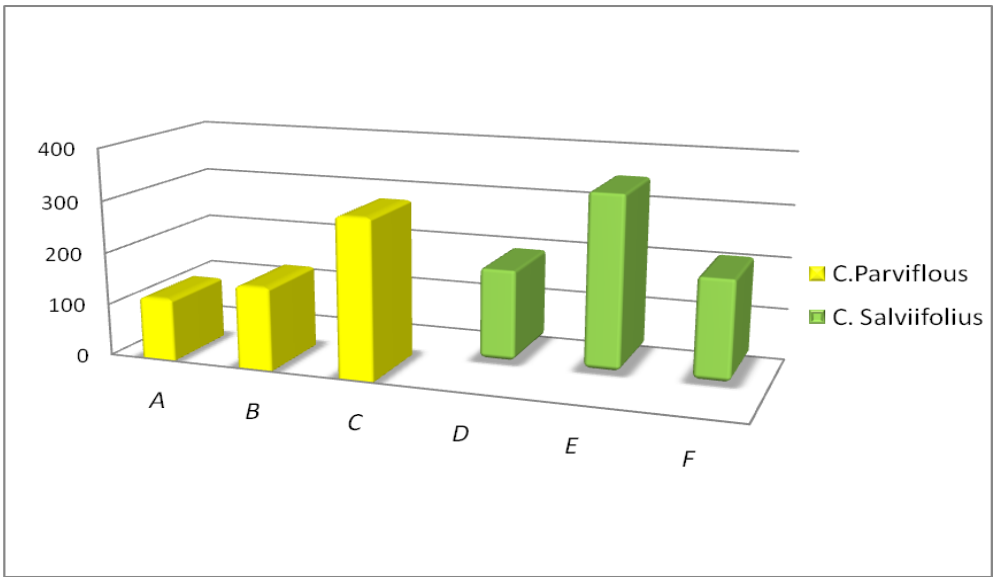

Figure.3 Total phenol contents of alcoholic extract s of leafs, steams and roots *C. parviflorus Lam* and *C. salviifolius L.*

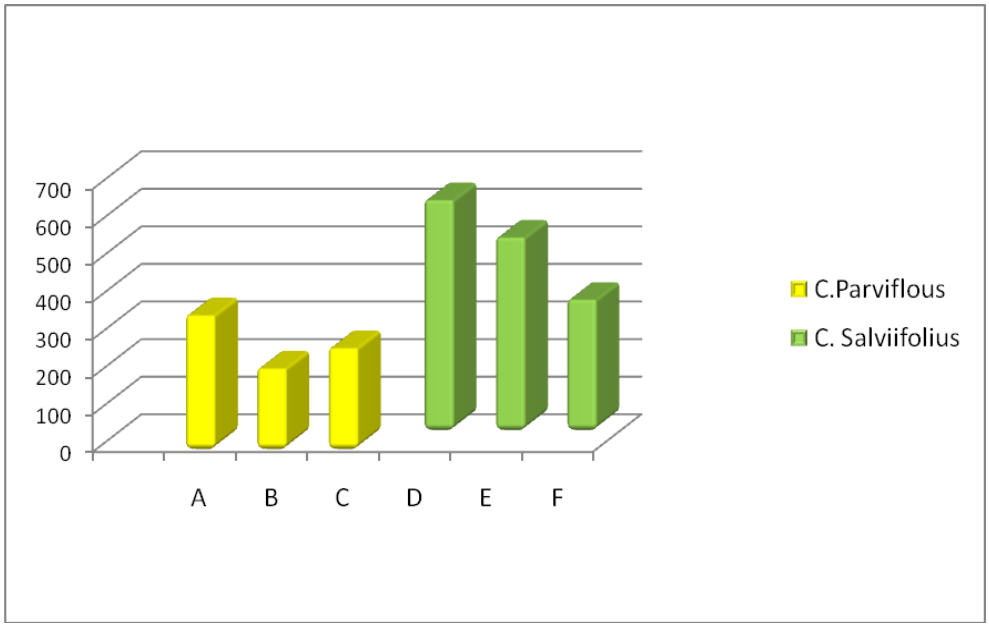

Figure.4 The inhibition zones of the studied extracts against *Escherichia coli*

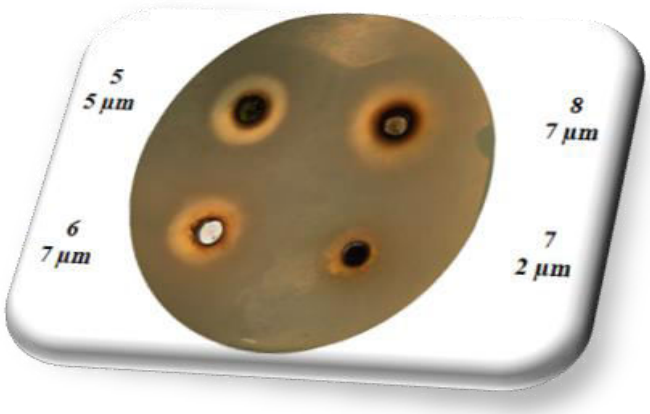

Figure.5 The inhibition zones of the studied extracts against *Escherichia coli*

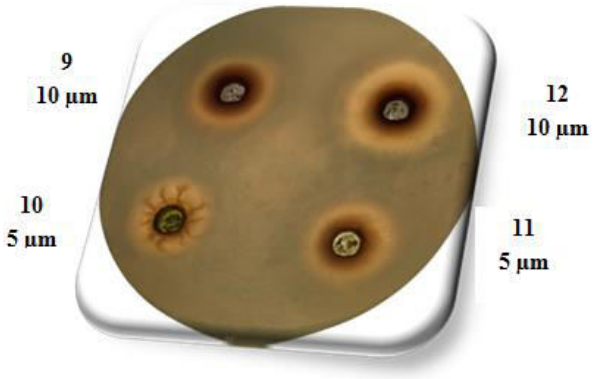

Figure.6 The inhibition zones of the studied extracts against *Pseudomonas*.

Figure.7 The inhibition zones of the studied extracts against *Bacillus cereus*.

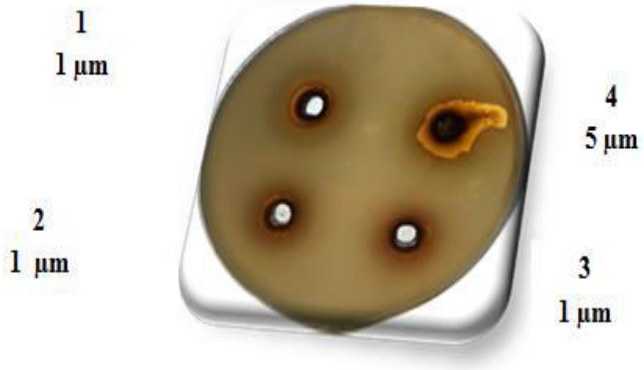

Figure.8 The inhibition zones of the studied extracts against *Bacillus cereus*.

Figure.9 The inhibition zones of the studied extracts against *Bacillus cereus*.

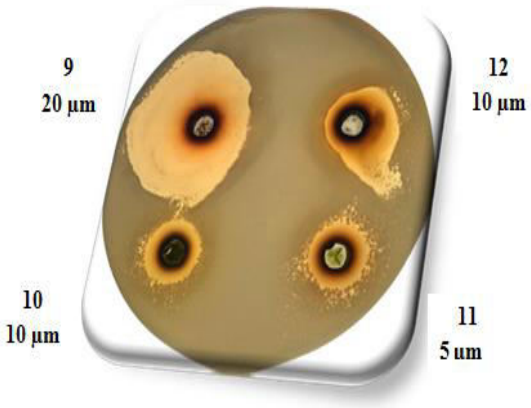

Figure.10 The inhibition zones of the studied extracts against *Streptococcus*.

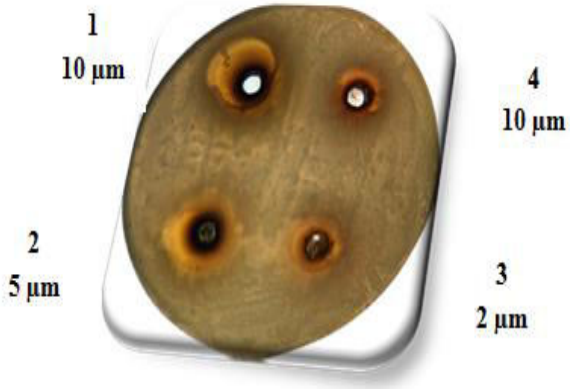

Figure.11 The inhibition zones of the studied extracts against *Streptococcus*.

Figure.12 The inhibition zones of the studied extracts against *Streptococcus*.

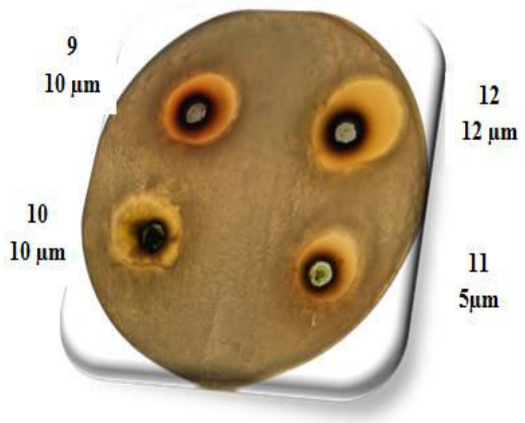

Figure.13 The inhibition zones of the studied extracts against *Fusarium.*

Figure.14 The inhibition zones of the studied extracts against *Fusarium*

Figure.15 The inhibition zones of the studied extracts against *Rhizopus*

Figure.16 The inhibition zones of the studied extracts against *Rhizopus*

Figure.17 The inhibition zones of the studied extracts against R*hizopus*

Streptococcus

The effect of aqueous and alcoholic extracts of the studied plants on the *Streptococcus* bacteria showed relative variations of inhabitation values, where the high values of inhibition zones were recorded for the root extracts of (15 and 12 µm) for the aqueous and alcoholic extracts of *C. salviifolius L*, respectively. The low value

of inhabitation zone of (2 µm) was recorded for the aqueous extract of *C. parviflorus Lam* roots. Meanwhile the steams extract of *C. parviflorus Lam* plant not gave any activity against the *Streptococcus* bacteria. The effect of the studied extracts on the *Streptococcus* were shown in the following Figures of (10-12).

Anti-Fungi activity studies

The anti Fungi activity studies were carried out on the aqueous and alcoholic extracts for leafs, steams and roots of the studied plants against some species of fungi. The results of anti-fungi tests are shown in Tables (10-11), and Figures (13 -), and described as following:

Fusarium

Tables (10 &11) showed the effect of different extracts of the studied plants extract against *Fusarium* fungi. The results showed that the inhibition zone recorded higher values of (5 -11 µm) for the alcoholic extracts of *C. salviifolius L*, while for *C. parviflorus Lam* plant no anti –fungi activities recorded for leafs and steams. On the other side the leafs of *C. parviflorus Lam* showed inhibitaion zone of value of (5 µm), (Tables 10 &11 and Figures of 13 -17).

Rhizopus

For the anti-fungi studies of *Rhizopus* fungi, the results recorded small variations of inhibitaion zones for most extracts of the studied plants, except for the alcoholic extract of *C. parviflorus Lam* roots which shown higher value of (15 µm), also all aqueous *C. parviflorus Lam* gave inhibition zones of (2 – 6 µm), except for roots of the same plant which not recorded any activity for *Rhizopus* fungi, Figures().

Discussion of antimicrobial studies

The diameters of growth inhibition zones exhibited by different extracts of against bacterial strains. As can be noted from this table, the ethyl acetate extract showed significantly the highest antibacterial activity against all pathogens bacteria, with a maximum inhibition zone of 20 µm and 20 mm against *Pseudomonas* and *Bacillus* for the aqueous extracts of leafs of the both selected plants in this study and roots of *C. parviflours* alcoholic extract. On the other hand, no significant results were recorded in alcoholic extract *C. salvifolius L* against *Pseudomonas*. In general, the gram-positive bacteria were found to have more susceptibility as compared to gram negative bacteria species. This result may be explained by the variation in chemical composition and structure of cell wall of both types of microorganisms. Thus, it is clear that the effectiveness of the extracts largely depends on the type of used solvent. It can be speculated that the extracts contained compounds with greater polarity than that of their counterparts present in extracts. This may be the likely explanation for significant differences in the microbial activity between the different compound extracts of the studied extracts.

The different tested extracts significantly reduced the colony diameters of the fungal pathogenic strains. The extracts of studied plants showed significant antifungal activity against *Rhizophus* comparing with the other fungi tested. However, the mechanism of the action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of the used solvent. All the exerts are more powerful inhibitory activity for *Aspergillussp*, also there is relative increase in anti –fungi activity of alcoholic extract as compared to aqueous extract for *Fusarium*. This observation clearly indicates that the existence of polarity residues in the extracts which have higher fungal abilities. According to several authors methanol extraction was more effective at antifungal activity than at water (Tsuchiya, 1996). This difference can be attributed to the origin of the different chemical composition between extracts; many studies have revealed a relationship between the chemical structure of phenolic compounds and their antimicrobial activity (Al Amiery, 2010).

Also the results of the antimicrobial activity studies were carried out on alcoholic and aqueous extracts for the studied plants of, *C. parviflorus Lam* and *C. salvifolius L* (leafs, steams and roots) against the selected bacteria and fungi have inhibitory effects on the growth of the studied bacteria and fungi, showed a difference in the effect of the extracts in the process of inhibiting microbial growth. Due to different polarity in the solvents (aqueous and Alcohol). It was also found that some of the extracts that showed the greatest inhibition against bacteria were similar in chemical composition as shown in the photochemical screening test. In this study the photochemical screening showed different types of compounds, this is may be explain the variations of the effects of extracts of the studied plants against the selected microbiological species (Iwu *et al.,* 1999).

The results were similar to some studies concluded the volatile compounds of plants reported that It has anti – microbial activities. Also, the result agrees with has been reported that plants which contain various phenolic compounds, such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids, glycosides, and glucosides contents have antibacterial activity (Naili, 2010).

According to the results recorded in this study which different two solvents (aqueous and alcoholic) were used to investigated the effect of extracts on phytochemical screening, anti-oxidant capacity, total phenols and phenolic acids, where the (leafs, steams and roots) of each plant were used in this study, the results showed that, there are wide variations in photochemical screening by comparing the aqueous and alcoholic extracts, also for leafs, steams and roots of the studied plants. The extraction of phenolic compounds depends on the type of the solvents, where the contents of the detected phenolic compounds showed variations in most of the used extracts.Also the studied plants containing important values of anti-oxidant capacity.

Also, the extracts were used for antibacterial anti-fungi activities, the results showed different effects on the selected pathogenic bacteria and fungi, also there is effect of the solvents of the studied extracts on the inhibition zones, the study revealed that due the effect of the polarities of the used solvents of (aqueous) and non aqueous solvent (alcohol).

Recommendations

Study the chemical composition of the medicine plants is still take place in man studies along the world due to the important of these plants for the treatments of many diseases especially for traditional therapeutic. Libya is one of some countries which containing specific medicine plants (endemic plants), therefore the study of chemical composition and their applications for anti-bacterial, anti-fungi, anti –filamentary anti-cancer and others very interesting and important.

Author Contributions

Hamad. M. Adress. Hasan: Investigation, formal analysis, writing—original draft. Najat. Ben. Areos: Validation, methodology, writing—reviewing. Naser. M. Emaza:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Al-Amiery, A. A. H. (2010). 'A study of the biological activities of *Avena sativa* extracts', pp. 31–34. https://doi.org/10.5897/AJPAC.9000004

Aronne G. and De Micco V. 2001. Seasonal dimorphism in the Mediterranean Cistus incanus L. subsp. incanus. Ann. Bot. (Lond.), 87(6): 789–794.

Alshammary, A. S. and Ibrahim. N. (2014). "Antimicrobial activity of *Citrulls colocynthis* extracts against Bacteria," Global Journal of Biology Agriculture and Health Sciences.3(4):71-73.

Arora, D and Kaur, J. (1999): Antimicrobial activity of spices, International Journal Antimicrob agents, 12: PP 257-262. http://dx.doi.org/10.1016/S0924-8579(99)00074-6

Balbaa. S. I., Hilal. S. H. and Zaki. A. Y, (1981): "Medicinal plant constituents" 3rd Ed. General Organization for University and School books.

Bouyahya, A., Abrini, J., Talbaoui, A., Et-Touys, A., Chatoui, K., Harhar, H., Bakri, Y. and Dakka, N. (2017). Phytochemical screening, antiradical and antibacterial activities of *Cistus crispus* from Morocco. J Mater Environ Sci, 8(5), PP1560-1566.

Burt, S. (2004): "Essential oils: Their antibacterial proper- ties and potential applications in foods", International Journal of Food Microbiology, 94,PP: 223-253.

Clauss. E. P. (1961): "Pharmacognosy", 4th Ed., 111 HeneryKimpton, London, pp:3.

Cosa, P., Vlietinck, A. J., Berghe, D. V., and Maes, L. (2006). Anti- infective potential of natural products.

How to develop a stronger in vitro proof- of - concept. J ethnopharmacol. 16.PP 290-302. http://dx.doi.org/10.1016/j.jep.2006.04.003

Egyptian Pharmacopeia (1984): General Organization for Governmental Printing Affairs, Cairo.

El Euch, S. K., Bouajila, J., and Bouzouita, N. (2015). Chemical composition, biological and cytotoxic activities of *Cistus salviifolius* flower buds and leafs extracts. *Industrial Crops and Products*,V 76,PP 1100-1105. http://dx.doi.org/10.1016/j.indcrop.2015.08.033

El Hifnawy, S. M.; Selim, M. A.; Seida, A. A. and Mohamed, M. I. [Eds] (1992): "Topics in Applied Pharmacognosy", Faculty of pharmacy, Cairo University, PP 66-69.

Ezeonu, C. S. and Ejikeme, C. M. (2016). Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. New Journal of Science, 2016. https://doi.org/10.1155/2016/5601327

Fang, X., Wang, R., Sun, S., Liu, X., Liu, X., Wang, W., and Okada, Y. (2018). Chemical constituents from the leafs of *Cistus parviflorus*. *Journal of Chinese Pharmaceutical sciences,* 27(1), 40-50. http://dx.doi.org/10.5246/jcps.2018.01.005

Guvenc¸. A., Yıldız, S., Özkan, A. M., Erdurak, C. S., Coşkun, M., Yılmaz, G., Okuyama, T., and Okada, Y. (2008). Antimicrobiological Studies on Turkish Cistus Species. *Pharmaceutical Biology,43(2),* PP178-183. http://dx.doi.org/10.1080/13880200590919537

Hasan, H. M., Ibrahim H. Habib1., Mariam. H. Gonaid and Mojahidul Islam. (2011). Comparative phytochemical and antimicrobial investigation of some plants growing in Al Jabal Al-Akhdar. J. Nat. Prod. Plant Resour., 2011, 1 (1):15-23.

Iwu, M. M, Duncan and C. O, Okunji (1999): New antimicrobials of plant origin. In J. Janick (ed). Prospective on new crops and new uses. ASHS press, Alexandria, V.A. pp: 457-462.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry, 47(10), 3954-3962. https://doi.org/10.1021/jf990146l

Li, J. W. and Vederas, J. C. (2009): Drug discovery and natural products: End of an era or an endless frontier Science, 325,PP 161-165. https://doi.org/10.1126/science.1168243

Mastino, P. M., Marchetti, M., Costa, J., and Usai, M. (2017). Comparison of essential oils from cistus species growing in Sardinia. Nat prod Res. 31(3).PP 299-307. https://doi.org/10.1080/14786419.2016.1236095

Mohamed, A. A., Khalil, A. A. and El-Beltagi, H. E. (2010). Antioxidant and antimicrobial properties of kaffmaryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). Grasas y aceites, 61(1), 67-75. http://dx.doi.org/10.3989/gya.064509

Muhaisen, H. M., Ab–Mous, M. M., Ddeeb, F. A., Rtemi, A. A., Taba, O. M., and Parveen, M. (2016). Antimicrobial agents from selected medicinal plants in Libya. Chinese journal of integrative medicine, 22(3),PP 177-184. https://doi.org/10.1007/s11655-015-2172-8

Mullen, W., Marks, S. C. and Crozier, A. (2007). Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. Journal of agricultural and food chemistry, 55(8), 3148-3157. https://doi.org/10.1021/jf062970x

Naili, M. B. (2010) 'Evaluation of antibacterial and antioxidant activities of *Artemisia campestris* (Astraceae) and *Ziziphus lotus* (Rhamnacea)', Arabian Journal of Chemistry., 3(2), pp. 79–84 https://doi.org/10.1016/j.arabjc.2010.02.002

Papaefthimiou, D., Papanikolaou, A., Falara, V., Givanoudi, S., Kostas, S., and Kanellis, A. K. (2014). Genus *Cistus*: a model for exploring labdane-type diterpenes' biosynthesis and a natural source of high value products with biological, aromatic, and pharmacological properties. *Frontiers in Chemistry,2, Article 35,* PP1-19. https://doi.org/10.3389/fchem.2014.00035

Singh, R. (2015). Medicinal Plants: A review. *Journal of plant Sciences*,3(1-1), PP50-55. https://doi.org/10.11648/j.jps.s.2015030101.18

Skorić, M., Todorović, S., Gligorijević, N., Janković, R., Živković, S., Ristić, M., & Radulović, S. (2012). Cytotoxic activity of ethanol extracts of in vitro grown *Cistus creticus* subsp. creticus L. on human cancer cell lines. Industrial Crops and Products, 38, 153-159. https://doi.org/10.1016/j.indcrop.2012.01.017

Tsuchiya, H. (1996) 'Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus'*, Journal of ethnopharmacology. Elsevier, 50(1), pp. 27–34. https://doi.org/10.1016/0378-8741(96)85514-0

UNESCO. (1996). Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 –

1997, Document CLT/DEC/PRO – Paris, France, 129.

Venugopal, R and Liu, R. H. (2012). Phytochemicals in diets for breast cancer prevention: The importance of resveratrol and ursolic acid. Food science Hum wellness. 1. pp 1-13. https://doi.org/10.1016/j.fshw.2012.12.001

Zeyaullah, M. Z., Ahmed, R., Nassem, A. Isalm, B., Hamad. M. Hasan., F. F. Benkhayal., Moshahid, A. Rizvi. and Arif. A. (2009). Catechol biodegraiation by Psedumonas Strain: a critical analysis. Int.j.chem.sci. No(7): 3: 2211–2221.

How to cite this article:

Hamad. M. Adress. Hasan, Najat. Ben. Areos and Naser. M. Emaza. 2024. Phytochemical Screening, Anti-bacterial and Anti-fungi Activities of Leafs, Stems and Roots of *C. parviflorus* Lam and *C. salviifolius* L Plants. *Int.J.Curr.Microbiol.App.Sci.* 13(11): 262-280. **doi:** https://doi.org/10.20546/ijcmas.2024.1311.029