PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF CERCIS SILIQUASTRUM

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Abstract:

The genus *Cercis* (redbud) belongs to the subfamily Caesalpinioideae of the plant family Fabaceae (Leguminosae). It includes 10 recognized species scattered widely across the warm, north-temperate zones of North America and Eurasia. *Cercis siliquastrum* is native to Lebanon found in altitudes ranging between 0-800m. The flowers are edible, with sweet to sour taste, used as salad garnish, and can be drunk as tea to relief stomach aches. Flowers are known to be rich in phytochemicals that are strongly associated with reduced risk of developing chronic diseases, such as cancer, diabetes, and cardiovascular diseases. Therefore, our aim of this study is to extract from these flowers' active phytochemicals. For this purpose, the flowers of *Cercis siliquastrum* were shade dried, then polyphenols were extracted using the chemical reagents methanol, ethanol, acetone,dichloromethane pre-treated with ammonia, and dichloromethane as a preparatory step. Then these extracts were used to determine the total phenolic content using Follin –Ciocalteu method and their antioxidant activity was assessed using DPPH radical scavenging activity. Whereas for the determination of the total flavonoids we used the Aluminum chloride method. And finally, the total terpenes were determined by Salkowski method. Descriptive statistics were performed in the present study; total phenol, flavonoids, and terpenes contents of the 5 extracts using solvents with different polarities were summarized as mean. To examine the relationship between total phenol, flavonoids and terpenes contents and DPPH radical

scavenging activity, Spearman's correlation coefficients were calculated between total phenol, flavonoids and terpenes contents and DPPH radical scavenging activity. Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 23. A p-value of < 0.05 was indicative of Statistical significance. Acetone was found to have the highest extraction capacity on phenols (4.825 mg GAE/100mg flower extract). And ethanol for total flavonoid (53 mg QE/100mg flower extract), dichloromethane showed to be the best in extracting terpene with capacity of (192 mg LE/100mg flower extract). In addition, the acetone extract exhibited the highest DPPH scavenging activity (IC50% 41.85 mg/mL). Un-significant negative strong correlations were observed between total flavonoid content and DPPH scavenging activity (r: -0.500 p: 0.391). Further investigation for the antioxidant activity should be performed to identify and isolate the bioactive metabolites responsible for this activity.

Keywords: *Cercis siliquastrum*, antioxidant activity, phenolic content, terpenes content, flavonoids content, DPPH scavenging activity

List of abbreviations

meGal	methylgallate
ROS	reactive oxygen species
H_2O_2	hydrogen peroxide
NIDDM	non-insulin dependent diabetes mellitus
IDDM	insulin dependent diabetes mellitus
PTB1B	protein tyrosine phosphatase 1B
PTPs	protein tyrosine phosphatase
IR	insulin receptor
AChE	acetylcholine esterase
BuChe	pseudocholinesterase
EtOH	ethanol
MeOH	methanol
BuOH	butanol
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DCMa	dichloromethane pretreated with ammonia
DCM	dichloromethane

DW dry weight

TPC total phenol content

TTC total terpene content

TFC total flavonoid content

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Chapter I. Generalities

I.1 Introduction:

Fabaceae also called leguminoseae, is a pea family of around 700 genera flowering plants. This family is in the order of Fabales and contains about 19,000 species, of which around 10 are Cercis L. (redbud) (XY. Lin, 2014). The species include shrubs, trees, vines, and herbs distributed in the northern hemisphere Europe, Eurasia, central Asia, eastern Asia (Li 1944; Davis *et al.,* 2002), northern America (A. Gray 1959; Isley 1975), and east of America (Davis *et al.,* 2002).

It is a genus of trees or shrubs of small to medium size with alternate, undivided, heart to kidney-shaped, edentate leaves with five to nine conspicuous veins radiating from their base (Everett, 1981), and having a pulvinus at both ends of the petiole. The fruits have a narrow flange of tissue (wing) on one side of the pod external to the placental strand (H. Jia and S.R. Manchester, 2014). Their flowers develop directly on the stem or trunk.

Some of these species are often multi-stemmed, with pointed leaves at their tips, and are light green in color such as C. *canadensis.* Others are round or vase shaped trees, with relatively thick, leathery, glaucous leaves such as C. *canadensis* var *texensis.* Some plants grow strictly upright and have glossy, dark green leaves such as C. *chinensis* (Coskun, 2003).

The flowers are reddish to pink, or white they blossom before the growth of the leaves, they offer an attractive view since they grow directly on the trunk (Raulston, 1968). Moreover, they are safe to eat and have sweet sour taste.

Figure 1: example of *Cercis siliquastrum* plant and flower (source: www.lebanon-flora.org)

The only remarkable species found in Lebanon is *C. siliquastrum* (Tohme *et al*., 2014).

To screen for new therapeutic agents in our foodstuff, and due to the complex chemical

composition and pharmacological diversity of the plant material, different C. siliquastrum flower extracts will be prepared using solvents of varying polarity. These extracts will be assessed for active phytochemicals, such as total phenolic content using the Folin-Ciocalteu method, total flavonoids by aluminium chloride assay and finally total terpenes according to the method of Salkowski. Finally, we will assess their antioxidant activity using DPPH radical scavenging.

I.2 Botanical description and distribution of the species C. *siliquastrum*

Family: Fabaceae (Leguminoseae)

Order: Fabales

Genus: *Cercis*

Species: *Cercis siliquastrum*

Common name: Redbud or Judas tree

Arabic name: زمزريق

Cercis siliquastrum known as Judas tree or redbud is distributed in the Mediterranean Region from France to Turkey and Afghanistan (Rechinger, 1986) and is distributed in the Lebanese mountains at an altitude (0-500 m) and up to 800 m (Rechinger 1986, Davies *et*

al., 2002) such as in Multaqa Anahyrayn; an area in the Shouf disctrict, where the plant material for the present work was collected. It is also found in 3 Lebanese natural reserves: (Figure 2) Bentael Nature Reserve, Horsh Ehden, Jabal Moussa Biosphere (Lebanon-flora.org).

Figure 2: *Cercis* geographic distribution (source: www.lebanon-flora.org)

1-Bentael Nature Reserve, 2- Horsh Ehden Nature Reserve, 3- Jabal Moussa Biosphere

A botanical description of *C. siliquastrum* was identified by Dr. Tanus el Hajj at Notre

Dame University, Faculty of Natural and Applied Sciences.

Cercis siliquastrum is a spreading, sometimes multi-stemmed tree grows up to 12 m. It has a relatively large number of first and second order branches Their heart-shaped leaves are often broader than long (5-10 cm high and up to 12.5 cm wide), heart-shaped at the base with a rounded or notched tip with usually seven main veins, dull green and glaucous on the upper surface, pale yellow from the end of October to November (Hopkins1942; Isley 1975).

The flowering season is between February and April, and flowers are up to 2 cm long, crimson, pink in the form of clusters that appear directly on twigs and trunks (cauliflory) before leaves shoot. They are pea- shaped, inflorescent, and have soft fragrance. And the fruits are brownish, 7-10 cm long flattery legumes, lasting throughout the winter (Raulston 1990; Robertson 1976, Everett 1981).

I.3 Phytochemical constituents of genus *Cercis*

The importance of the genus Cercis in China and India, in addition to certain Arab Regions such as Iran and Palestine in its uses in traditional medicine; and in the edibility of its flowers and fruits, this led the researcher to investigate the bioactive compounds of all the parts of the plants. It was found that all have biological activities and can be used

according to the literature. The most extracted phytochemicals are phenolic compounds like flavonoids, phenolic acids, anthocyanins, stilbenes, lignans, also carotenoids, cyanogenic glycosides, and chlorophyll are found (Zang *et al.,* 2005 and 2006; Na *et al.,* 2009; Li *et al.,* 2005).

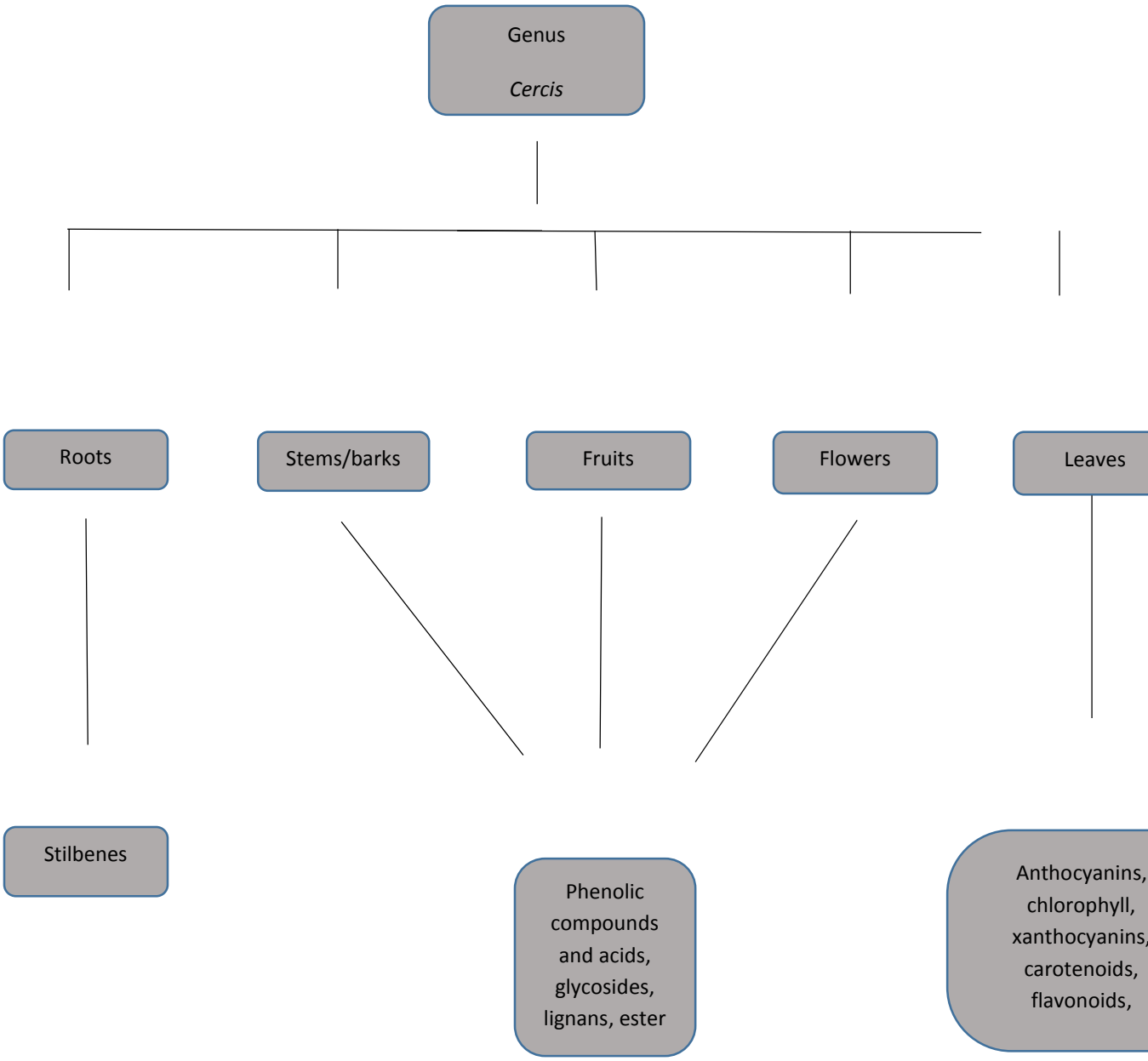


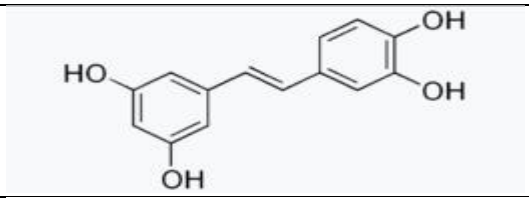
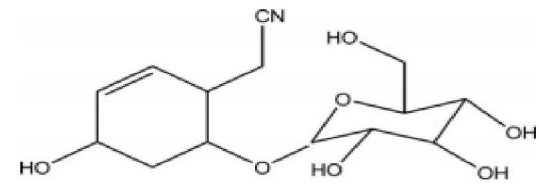
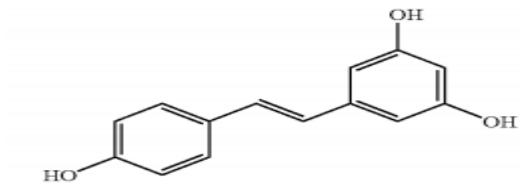
Figure 3: Major constituents of the *Genus cercis* from different parts of different species

I.3.1 Phenolic Compounds in *Cercis genus*

Phenolic compounds are the major bioactive compounds extracted from different parts of Cercis plants, and flavonoids being the most abundant phenolic compounds in this genus.

They are secondary metabolites found in plants. They possess one or more aromatic rings with one or more hydroxyl groups. They are categorized into flavonoids, coumarins, phenolic acids, stilbenes, and tannins. They provide several essential functions in the reproduction and growth of the plants, such as defending against parasites, predators, and pathogens, as well as giving the plants their color. In addition to that, they play a vital role in human health such as reducing the risk of chronic diseases (Wu and Liu *et al.,* 2002).

Table 1: Phenolic compounds in *Cercis genus*

Phytoconstituents	**Structure**	**Species**	**Reference**
Piceattanol (stillbenoid)		*C. chinensis Bunge*	Cardona *et al.* 1986
Menisdaurin		*C. chinensis Bunge*	Nakanishi *et al.* 1994
Reservatrol		*C. chinensis*	Min *et al.,* 2010
Daucosterin		*C. chinensis Bunge*	Wu *et al.*1979

I.3.1.1 Flavonoids in *Cercis genus*

Flavonoids are the largest group of secondary metabolites (around two thirds of phenolics) that have been identified in fruits, vegetables, and the leaves of herbal plants.

They are usually added to nutraceuticals as antioxidants. They can significantly reduce damages caused by cardiovascular diseases and cancer (Erlund, 2004; Jeon *et al.,* 2001).

They have a nuclear structure of C6–C3–C6, comprising two benzene rings connected by a pyrene ring containing oxygen. Differences in the generic structure of the heterocycle ring or, C ring classify them as flavonols, flavones, flavanols (catechins) flavanones, isoflavonoids, and anthocyanidins (Atmani *et al.,* 2009; Heim, Tagliaferro, & Bobilya, 2002; Hollman *et al.,* 2000).

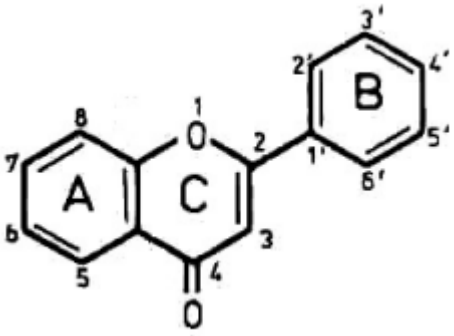
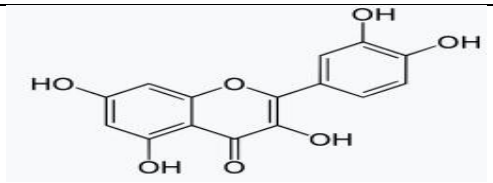
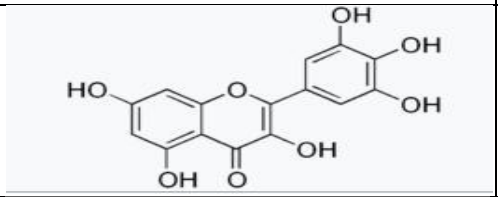
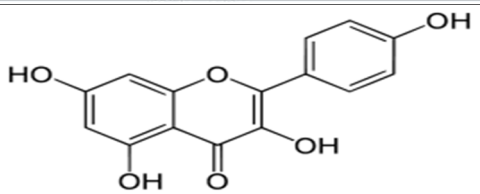
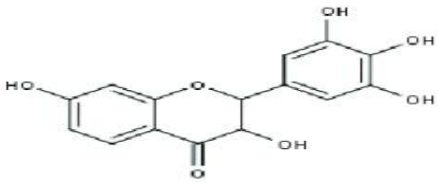
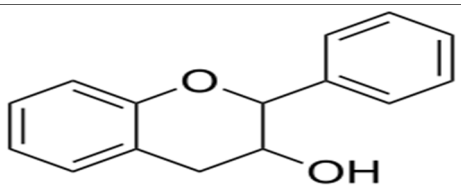
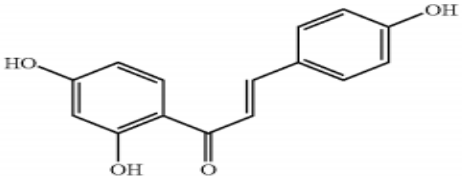
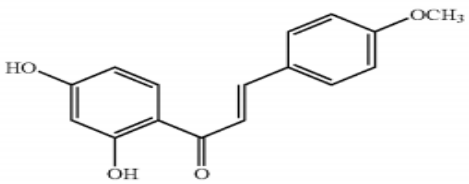
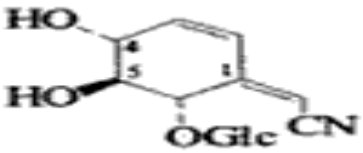
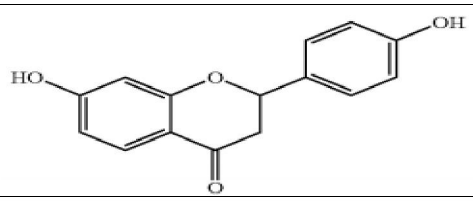
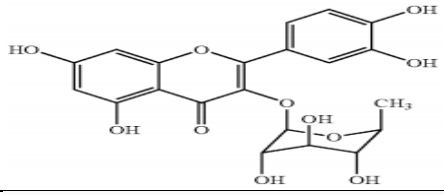
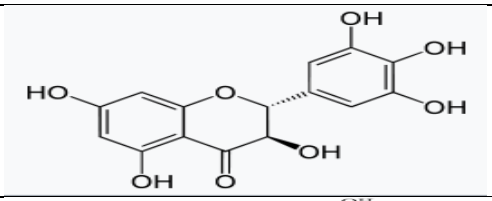
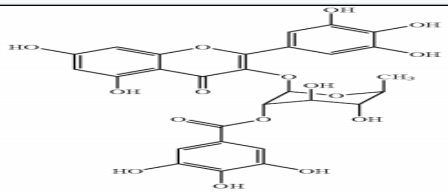
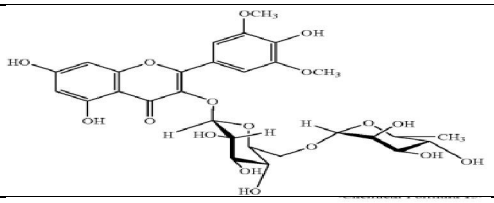
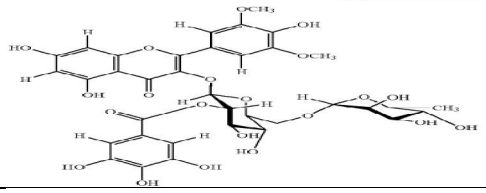
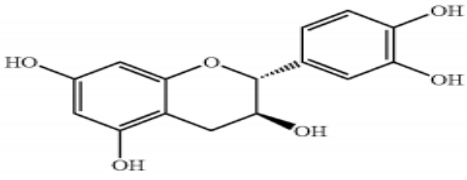


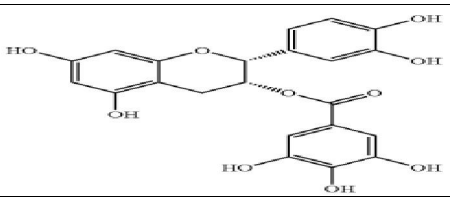
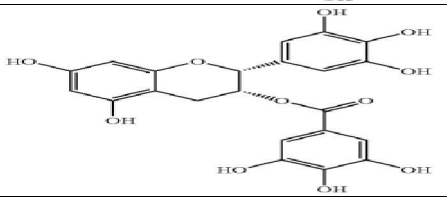
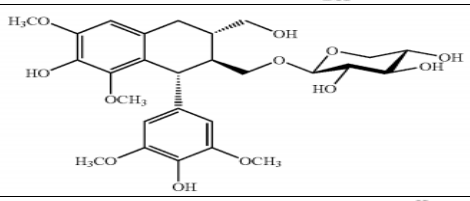
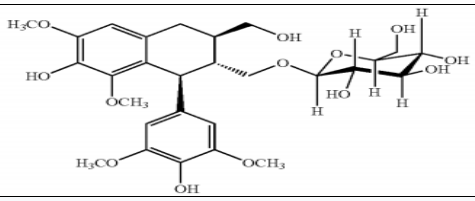
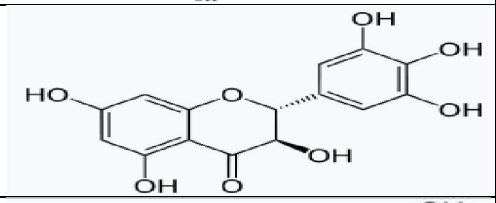
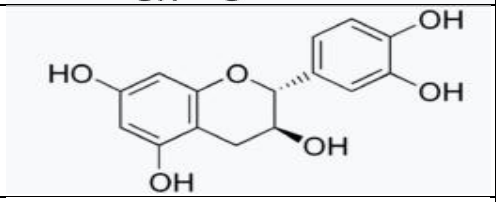
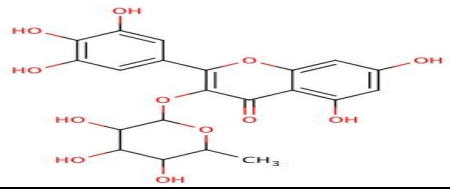
Figure 4: The basic nucleus of flavonoids (Stace 1980).

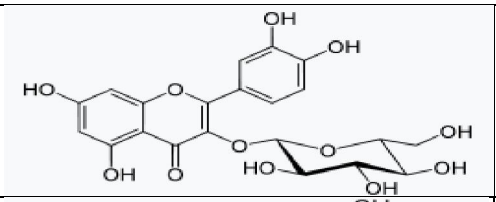
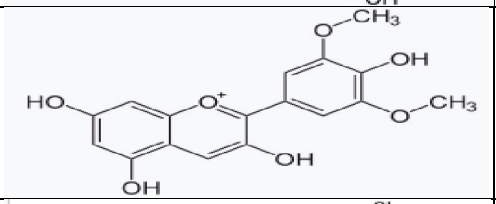
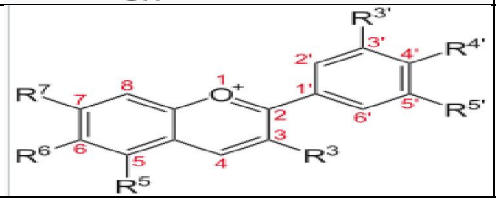
Some flavonoids were extracted from Cercis species, they are displayed in table 2.

Table 2: Flavonoids from various Cercis species

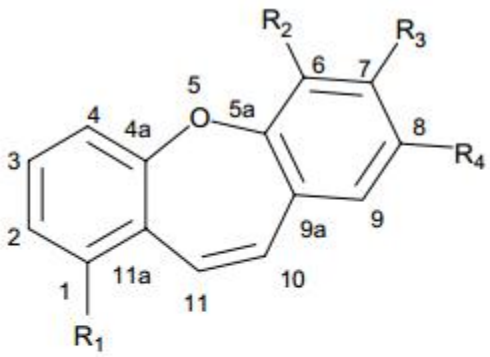
Phytoconstituents	Structure	Species	References
Quercitrin		*C.chinensis;* *C. siliquastrum;* *C. canadensis*	Ling *et al.* 2009; Yang *et al.* 2015
Myrecitin		*C. chinensis;* *C.. siliquastrum;* *C. canadensis*	Ling *et al.* 2009; Yang *et al.,* 2015
Kaempferol		*C. chinensis;* *C. siliquastrum;* *C. canadensis*	Ling *et al.* 2009; Yang *et al.,* 2015
Dihydrorobinetin= deoxymyricetrin synonyms (flavonoid)		*C. chinensis*	Manners and Jurd, 1979
Flavan-3-O gallate		*C.chinensis*	Nonaka *et al*, 1983
2',4'4-Trihydroxychalcone(Isoquiritigenin		*C. chinensis*	Min *et al.,* 2010
2',4'Dihydroxy-4-methoxychalcone		*C. chinensis*	Min *et al.,* 2010

Lithospermoside (biflavonoid)		*C. chinensis Bunge*	Wu *et al.* 1979
Liquiriteginin		*C. chinensis*	Min *et al.,* 2010
Quercetin-3-O-a-L-rhamnopyranoside(Quercitin)		*C. chinensis*	Min *et al.,* 2010
Dihydromyricetin (flavonoid)		*C. chinensis* Bunge	Shen *et al.* 1993
Myricetin-3-O-(2'-O-galloyl)-a-L-rhamnopyranoside		*C. chinensis*	Min *et al.,* 2010
Syrinngetin-3-O-rutinoside		*C. chinensis*	Min *et al.,* 2010
Syringetin-3-O-(2"-O-galloyl)-ritinoside		*C. chinensis*	Min *et al.,* 2010
(+)-Catechin		*C. chinensis*	

(-)-Epicatechin-3-O-gallate		*C. chinensis*	Min *et al.,* 2010
(-)-Epigallocatechin-3-O-gallate		*C. chinensis*	Min *et al.,* 2010
(-)-Lyoniresinol3a-O-b-D-xylopyranoside		*C. chinensis*	Min *et al.,* 2010
(+)-Lyoninesinol-3a-O-b-D-glucopyranoside		*C. chinensis*	Min *et al.,* 2010
Dihydromyricetin		*C. chinensis Bunge*	Shen *et al.* 1993
Catechin		*C. chinensis Bunge*	Shen *et al.* 1993
Myricetin-3-rhamnoside		*C. siliquastrum*	Harborne *et al.*1971
Myricitoside C		*C. siliquastrum*	Valan *et al.*2010

Quercetin-3-glucoside = (isoquercitin)		*C. Canadensis*	Harborne, 1967; Douglass *et al.* 1949
Malvidin		*C. siliquastrum*	Torck *et al,*1969 ,Sagareishvili and Ananiya 1990
Anthocyanins		*C. Canadensis*	(Choinski *et al.* 2003; Cai *et al.*2005)

Other phenolic compounds extracted from *C. chinensis* Bunge



	R1	R2	R3	R4
1	H	OMe	Me	OH
2	OMe	OH	Me	OMe
3	OH	OMe	Me	OMe
4	OH	OH	Me	OMe
5	OH	OMe	Me	OH

Where:

1) 6-methoxy-7-methyl-8-hydroxydibenz[b,f]oxepin (Zhang *et al.,* 2006)
2) 1,8-dimethoxy-6-hydroxy-7-methyldibenz[b,f]oxepin (Zhang *et al.,* 2006)
3) 1-hydroxy-6,8-dimethyoxy-7-methyldibenz[b,f]oxepin (Zhang *et al.,* 2006)
4) Pacharin (Anjaneyulu *et al.,* 1984)
5) Bauhiniastatin 4 (Petit *et al.,* 2006)

I.3.1.2 Other Phenolic compounds in *Cercis genus*

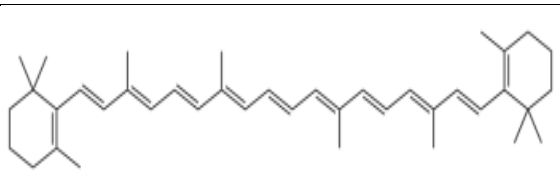
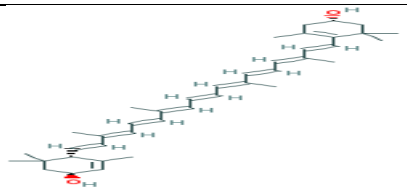
Other Phenolic compounds were extracted from flowers, stems/barks, roots, and fruits in Cercis genus. They were stilbenes such as stillbenoids, phenolic acids, esters, lignans, glycosides, sterols such as β- sterol, and chlorophyll (Liu *et al.*, 2002).

I.3.2 Carotenoids in *Cercis genus*

Carotenoids occur widely in plants, animals, and microorganisms. They are natural pigments with both pro-vitamin and antioxidant roles. They give the yellow-orange color of fruits and vegetables. They have a 40-carbon skeleton of isoprene units that can be cyclized at one or at both ends. They play an important role in photosynthesis and photo-protection in plant tissues.

Photo-protection is associated in the antioxidant activity in human health, since they react with free radicals especially in the lipid soluble environment. Sufficient concentrations of carotenoids play a preventive role in lipid oxidation and related oxidative stress (Britton, 1995). Table 3 shows carotenoids extracted from *Cercis genus*.

Table 3: Carotenoids from various *Cercis* species

Phytoconstituents	**Structure**	**Species**	**Reference**
Carotenoids		*C. Canadensis*	Huges *et al.* 2007
Xanthophyll		*C. Canadensis*	Krause *et al.* 1995; Barker *et al.* 1997

I.4 Traditional uses of *Cercis* species

Cercis species (Leguminosae) have been used in traditional medicine for thousands of years. They are known to be used for detoxification, and some believe that the red color

of flowers is correlated with the treatment of blood diseases. The biomass is used for cooling blood, wind cold dampness arthralgia, amenorrhea, rabies, and is thought to cure rheumatism (Yuan, 2006). Whereas the barks are used as folk remedy for leukemia, for restoring menstrual flow, blood gas pain, traumatic injury, sore throat, insect, and snake bites (Wang *et al.,* 2004). The flowers are used to treat rheumatic ache, stomachache, and nasal abscess. The fruits are used for coughs (Xie *et al.,* 2005). Whereas the stem and roots, are believed to have antioxidants, anti-microbial, anti-fungal, anti-inflammatory activities (Kim *et al.,* 1995; Na *et al.,* 2009).

Cercis siliquastrum, mostly the bark, has been used in traditional Mediterranean medicine as herbal remedies for antidiarrheal and gastric anti-inflammatory (A. Shatayeh, 2002, 2003).

Moreover, C. siliquastrum has insecticidal activity against *Frankliniella occidentalis* which is a pest that causes damage to a vast range of crops such as ornaments, onion, tobacco, and beans (Tommasini and Maini, 1995).

The biomass of *C. chinensis* is used as raw drug in the Chinese Ayurveda and homeopathy and is one of the Chinese Materia Medica (Shi *et al.,* 2018).

I.5 Research reports on pharmacological studies of genus *Cercis*

The importance of *C. chinensis* in traditional medicine leads to the investigation of all other species in the genus *Cercis.*

C. chinensis is the most studied specie in this genus. Whereas few studies have been done on *C. Canadensis* and *C. siliquastrum*. The three species are often described in the Pharmacopeias and monographies.

The whole plant of *C. siliquastrum* is listed in the WHO monographies, in the Pharmacopoeia of the United States, as well as the Indian Pharmacopeia (Kumar, 2014).

Various pharmacological studies have been reported from different *Cercis* species and they are summarized in Table 4. However, in the present work, we are interested to obtain whether *Cercis siliquastrum* flowers possess antioxidant activity.

Table 4: Pharmacological antioxidant and anti-diabetic properties of different *Cercis species*

Plant name	**Part of the plant**	**Identified compounds**	**Pharmacological properties/use**	**References**
C. Canadensis	Inner bark	NA	Diarrhea and leukemia	Indiana Medica
C. Canadensis	Seed pods	Isoquercitn	Antioxidant and/or antiradical activity	Prochazkova *et al,* 2011
C.. Chinensis	Leaves	Myrecitrin, quercetin, afzelin	Antioxidant, anti-tumor, hepatopretective	Ling *et al,* 2009 Yang *et al,*
		Methygallate (meGAL)	Antioxidant	Whang *et al,* 2005
C. chinensis	Stems	Dibutyl phthalate	Pesticide	Peng *et al,* 2014
		Phthalic acid, Isobutyl nonyl ester	Anti-inflammatory Anti-tumor, Cure chronic cardiovascular and cerebrovascular diseases	Huang *et el,* 2006

			Anti- bacterial	
		4, 6-di-Omethyl-alpha-d-galactose	Nutritive sweetener	Aparana *et al,* 2012
		Phytol	Precursor of Vit.E and K1	T. Netscher, 2007
		n-hexandecanoic acid	Anti-inflammatory	Aparana *et al,* 2012
C. chinensis Bunge	Roots	Naphthaquinone	Allelopathic	Babula *et al*,2009
		5-hydroxy-1,4,naphthaquinone	Antimicrobial	Lim *et al,* 2007
		Flavonol glycoside	Antioxidant	Na *et al,* 2009
		Stilbene	Antimicrobial	Li *et al,* 2005
C. chinensis	Bark	NA	Anti-inflammatory, Analgesic	Zhang *et al*. 2011
C.chinensis	Flowers	D-pinitol	Anti-inflammatory	R.K. Singha *et al*.2001;
			Anti-diabetic	Ajuah *et al,* 2000
			Antioxidant	B.Orthen *et al,* 1994
			Immuno-regulator	S. Prashant *et al,* 2011

C. chinensis	NA	Reservatrol	Cardio-protective	F. Brizdelli *et al,*2009
			Antioxidant Anti- inflammatory	J. Chung *et al,* 2012
			Anti-diabetic Anti-obesity	J. Jeong *et al,* 2012
C. chinensis Bunge	Heartwood	Naphthalenedione	Larvicidal activity	Lim *et al*. 2007
C. siliquastrum	NA	Myricitoside C	Anti-hepatotoxic activity	Valan *et al.* 2010
C. siliquastrum	Leaves	Myricetin	Antimalarial activity	Kaiser *et al.* 2007
C. siliquastrum	Roots	NA	Stomach problems	Brussel, 2004

In this study, the phytochemical composition and antioxidant activity will be tested.

I.5.1 Antioxidant activity of *Cercis genus*

Antioxidants are often described as "free radical scavengers" meaning that prevent the free radicals from taking electrons from other molecules. They play a key role in the body defense system against reactive oxygen species (ROS) which is associated with many diseases such as: impaired immune system and increased risk of infectious diseases, cancer, insulin dependent diabetes mellitus, non-insulin dependent diabetes mellitus, autoimmune diseases such as rheumatoid (Hsieh et al, 2004;Finkel and Holbrook,2000; Lee et al, 2002), eye diseases including cataract and retinal damage leading to age-related macular degeneration, various respiratory diseases, ankylosing spondylitis, Schizophrenia, AlZheimer's disease, and coronary heart diseases (Hill *et al*,1993; Beckman and Ames, 1998; McCord, 2000).

Free radicals are highly reactive chemical species that may increase during physical exercise; they can exist independently since they contain one or more unpaired electron in their outer orbit (Maxwell, 1995; Sen, 1995; Dekkers *et al.* 1996). During oxidative metabolism around 4-5% of the oxygen consumed are transformed into free radicals; whereas the rest amount of oxygen is bound to hydrogen and thus is reduced to water through a process called oxidative phosphorylation. This process requires 4 steps and the

generation of H_2O_2.These equations are called oxygen-derived intermediates (Yu, 1994).

H_2O_2 is not considered a free radical by itself since it does not contain unpaired electrons; however, it's considered a reactive oxygen species due to its high reactivity with reactive transition metals that lead to the formation of reactive oxygen species (ROS). Moreover, ROS can also be formed due to long sun exposure, stress, activated leukocytes as part of immune response, and normal cellular respiration.

The body can produce antioxidants naturally in situ to block or counteract the ROS, however they are not sufficient alone. Therefore, they must be supported by consuming supplements or fruits and vegetables that are rich in antioxidants. These antioxidants act as "free radical scavengers" by preventing and repairing damage caused by ROS thus reducing the risk of development of cancer and other chronic diseases and enhancing the immune system. (Hasler *et al.*1999 ; Valko *et al.* 2006 ; Parthasarathy *et al.*1999 ; Frei, 1997 ; Chatterjee *et al.* 2007).

Na *et.,al* in 2010 conducted a study on *C. chinensis* . They prepared an extract from the stems and leaves using 60% ethanol aqueous solution as a solvent at room temperature for two weeks. Then the 60% ethanol extracts were suspended in water and partitioned with hexane leading to the production of hexane fraction and residue. Then the resultant residue

was partitioned using ethyl acetate and thus leading to the production of ethyl acetate fraction and a residue. And furthermore, the resultant residue was partitioned with butanol and thus yielding butanol fraction and residue. Ethyl acetate and butanol fractions were compared to vitamin E as a control group.

EtAc and BuOH fractions were separated from other fraction using gel Column Chromatography and then 20 chemical compounds were obtained using HPLC. These compounds were: 2',4',4-Trihydroxychalcone (I soliquirtigenin), Methyl gallate, Gallic acid, Piceattanol, Ethyl gallate, Liquiritigenin, Reservatrol, Myrecitin, Myrecitin-3-O-α-L-rhamnopyranoside , Myrecitin-3-O-(2'-O-galloyl)-α-L-rhamnopyranoside, Afzelin, Catechin, (-) Epicatechin-3-O-gallate, (-)-epigallocatechin-3-O-gallate, (+)-Lyoninesinol-3a-O-β-D-glucopyranoside, (-)-lyoniresinol-3a-O-β-Dxylopyranoside, Syringetin-3-O-(2"-O-galloyl)-rutinoside, Syringetin-3-O-rutinoside, Quercitin-3-O-α-L-rhamnopyranoside (Quercitin), 2'4'-Dihydroxy-4-methoxychalcone. It has been shown that all 20 compounds have antioxidant activity , however; Syringetin-3-O-(2"-O-galloyl)-rutinoside was confirmed to be the novel compound.

Whang *et al.,* in 2005 studied *C.chinensis* leaves. Leaves were extracted by methanol solvent, then the methanol extract was suspended with water and then partitioned with ether using column chromatography. A pure compound meGal was extracted and identfied using

differnet spectroscopic parameters: FTIR, FAB-MS, H-NMR, and C-NMR.

Then human umbilical vein endothelial cells were treated with three different concentrations 0/02, 0.1, 0.5 mM of meGal for 1 hour prior to the addition of 1 mM of H_2O_2. Results showed that meGal has antioxidant effect especially for the long term exposure (48hr) at low concentrations (0.1mM) of H_2O_2.

Also, another study was conducted by Shi et al., in 2018 on the leaves of C. chinensis for the extraction of flavonoids (Myricitin, Quercitin, and Afzelin). Leaves were dissolved in a 70% methanol/water solution. Then the sample was extracted using ultrasonic extraction and filtered by organic micrporous membrane. Then the filtrate was used as the sample solution. And then separated using column chromatography at 30°C and detected under UV detector at wavelength=254nm. It is already known that the above-mentioned flavonoids have antioxidant activity, however the extraction using this method ionic liquids yielded 3-5 times higher extracts than using the traditional methods.

Dauglass *et al.,* 1949 were able to isolate isoquercitin from dried seed pods of *Cercis Canadensis* in both classical ways and paper partition chromatography. In the classical way, seed pods were immersed in 95% ethanol/water, and then boiled and strained with a cloth. Bright yellow crystals of isoquercitin were finally obtained by the method of Sando

and Barlett.

Whereas the paper partitioning chromatography, several solvents were used to isolate the pigment, such as: 40% butanol, 10% acetic acid, m-cresol, and water. Then the isolated pigment was chromatographed alone with a known standard of isoquercitin. The obtained chromatographic reagents from the pigment were identical to those of isoquercitin.

The seed pods yielded 0.0076% isoquercitin, and it is believed that it has antioxidant activity (Prochazkova *et al,* 2011).

I.5.2 Anti-diabetic activity of *Cercis* species

Diabetes is a group of metabolic diseases characterized by high levels of blood sugar (hyperglycemia). Diabetes has four main types: type 1 diabetes, type 2 diabetes which is a non-insulin dependent diabetes mellitus, drug-induced or chronic pancreatitis-induced diabetes, and gestational diabetes. The imbalance between the rate of generation and rate of elimination of reactive oxygen species is closely associated with the development of type1 and type 2 diabetes. However, diabetes type2 accounts for 90% of diabetes cases worldwide; and it is expected to duplicate by 2030 World Health Organization (WHO, 2000). Diabetes type 2 is a chronic disease where insulin secretions and/or sensitivity start decreasing gradually. On the long term, complications start to form hyperglycemia which

leads to an increased risk of heart stroke (myocardial infarction), amputation, dysfunction, and failure of different organs especially the eyes (diabetic retinopathy), kidney failure (diabetic nephropathy), nerves (diabetic neuropathy), and blood vessels(atherosclerosis) (Diabetes Care, 2010). Different modern medications have been used as hypoglycemic drugs, but they showed undesired side effects; therefore, an alternative approach such as medicinal plants, herbal drugs, and healthy diet showed effectiveness and safety. Thus, it is suggested that replacing saturated fats and trans-fatty acids by polyunsaturated fats may reduce the risk of type 2 diabetes and obesity by affecting the insulin sensitivity (Oktayoglu et al., 2009; 2010; Tesfaye et al., 2011; Chintan et al., 2011; Valko et al., 2007).

Protein tyrosine phosphatase 1B (PTP1B) is a member of the protein tyrosine phosphatases family that are important for regulating signaling events in the cellular pathway and is considered as a negative modulator of insulin receptors. Many secondary metabolites such as flavonoids, coumarins, lignans, and terpenes act as PTP1B inhibitors (Zang *et al.,* 2007; Alonso *et al.,* 2004; Lessard *et al.,* 2010; Combs, 2010).

I.5.3 Neuroprotective activity of *Cercis* species

Cholinesterase is powerful hydrolytic enzyme that regulates the neurotransmitter

acetylcholine in its postsynaptic action. It includes two types: acetylcholinesterase (AChE) which is the principal hydrolytic enzyme found the excitable tissues such as brain, nerves, red blood cells membranes, and skeletal muscles; and pseudocholinesterase (BuChE) that is found mainly in the liver; however, it can functionally replace (AChE) when the latter is extremely suppressed (Colovic et al.2013; Lionetto et al. 2013).

Cholinesterase inhibitors increase the level and length of Ach action, however based on their mode of action they are classified into three types:

1- reversible inhibitors (carbamate, quaternary or tertiary ammonium group) that are the least dangerous and used for therapeutic purposes such as (AlZheimer disease, myasthenia gravis, post-operative ileus, underactive bladder, glaucoma, antidotes, and anti-cholinergic overdose)

2-irreversible inhibitors with intermediate toxicity and used mainly as agricultural pesticides.

3- quasi-irreversible (pseudo-irreversible) and are the most dangerous inhibitors used in wars as chemical weapons and pesticides and can be classified as weak/short acting and strong/long acting (Giacobini 2000; Aggarwal and Zimmern 2016; Jiang et al. 2017; Colovic et al. 2013; Shaikh et al. 2014).

The reversible inhibitors of AChE are very important in the treatment of AlZheimer disease since it is a neurodegenerative disease that affects people at the age of 65 and above, it is characterized by cognitive defect, memory loss, and behavioral impairment since this disease caused by a decrease of acetylcholine due to cholinergic neurons in special parts in the brain such as (cholinergic hypothesis) cortex and hippocampus (Terry and Buccafusco 2003; Selkoe 2001).

I.6 Food recipes of *Cercis* species

Roots of C. siliquastrum are boiled like tea and drunk for treating stomach problems. Whereas the flowers are edible raw and used on fruit salads as garnish, or eaten pickled by Indians (Brussel 1201, Brussel 1366).

2. Material and Methods

2.1 Reagents and standards

Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), gallic acid, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, (+)-catechin, ethylene-diamine-tetra-acetic acid (EDTA), 3-[2-Pyridyl]-5,6-diphenyl-1,2,4-triazine-4,4′-disulfonic acid monosodium salt hydrate (Ferrozine), β-carotene, linoleic acid, and Tween-40 were purchased from Sigma-Aldrich Co (Steinheim, Germany); $FeSO_4 \cdot 7H_20$ was purchased

from Merck. Ultra pure water was used for ferrous ion chelating assay. All other reagents and organic solvents used were of analytical grade.

2.2 Plant material

The flowers of Cercis *siliquastrum* were collected in March 2017 from Multaqa al Nahrain in the Chouf region of Mount Lebanon (altitude 0-200 m). The plant was identified by Dr. Tanios al Hajj (NDU, Lebanon). The flowers were shade dried and pulverized using an electric blender to increase contact between the plant material to be extracted and the liquid (solvent). Then powdered flowers have been macerated in five different solvents (MeOH, , EtOH, Acetone, Dichloromethane, and Ammonia-dichloromethane) to reach the desired solubility and use of the extraction.

2.3 Extraction Method

For the preparation of MeOH, EtOH, acetone, and dichloromethane extracts, 20 g of plant powder were macerated in 200 mL of solvent under constant magnetic agitation for 24 h at room temperature. The mixture was then filtered and condensed at 40 °C under reduced pressure.

Whereas for the preparation of ammonia-dichloromethane extract, 20g of plant powder were moistened for 1h under the hood with NH_4OH solution then (200mL) of dichloromethane were added. After that the mixture was macerated for 24 h under magnetic stirring and then filtered. An organic phase was obtained and concentrated at 40

°C under reduced pressure.

All mixtures were then freeze dried using IKA RV 10 BASIC rotary evaporator. The obtained extracts were kept in a cool place in dark containers.

2.4Total Phenolics, Total Flavonoids, and Total Terpenes Content

Total phenols in the extracts were assessed by a modified Folin-Ciocalteu method (Koivikko *et al*., 2005). First (0.5 ml) of distilled water was added to extracts (different volumes) then mixed with (0.5 mL) of 1N Folin-Ciocalteu reagent. After 3 min, the mixture was neutralized with 1 mL of 20% (w/v) sodium carbonate (Na_2CO_3). After incubation in the dark at room temperature for 45 min, mixtures were centrifuged (8 min at 2800 *g*) and absorbance was measured at 730 nm versus a prepared blank using a Jenway 6405 UV/Vis spectrophotometer.

Total phenol content was expressed as mg of gallic acid equivalents (mg GAE) per 100 mg of extract. All measurements were performed in duplicates.

Total flavonoids content was determined by a modified aluminum chloride method (Erden *et al.,* 2015). In a 5ml volumetric flask, (2ml) of distilled water was added to (0.5ml) of sample extract.Then (150 µL) of 5% (w/v) sodium nitrate ($NaNO_{2)}$ solution were added and mixed and then (150 µL) of 10% (w/v) aluminium chloride ($AlCl_{3)}$ was added and mixed. After 5 min, (1ml) of 1M sodium hydroxide (NaOH) was added and mixd. And

after 1 min distilled water was added to the mark. And then immediately the absorbance of the colored flavonoid-aluminium complex was measured at 510nm using UV-visible spectrophotometer versus a blank.

Total flavonoid content expressed as mg of quercetin equivalents (mg QE) per 100 mg of extract. All measurements were performed in duplicates

Test for terpenoids (Salkowski test): 5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids (Edeoga *et al.,* 2005)

The total terpenoid content of the plant extracts was determined based on an assay described by (Ghorai *et al.* 2012) with some modifications. Linalool was used as the standard for estimation. An aliquot of the reaction mixture obtained after Salkowski test employed for the qualitative analysis of terpenoids in the extract was transferred to colorimetric cuvette. The absorbance was measured at 538 nm against blank i.e., 95% (v/v) methanol. For the standard curve, 200 µl of linalool solution in methanol was added with 1.5 ml chloroform and serial dilutions [dilution level-100 mg/200 µl to 1 mg/200 µl linalool Conc.] were prepared in which total volume of 200 µl was made up by the addition of 95% (v/v) methanol. Calibration curve of linalool was plotted and the total terpenoids content expressed as milligrams of linalool equivalents per gram of dry weight (mg linalool/g DW)

was determined using the regression equation. Samples were analyzed in duplicates.

2.5 Measurement of antioxidant activity DPPH radical scavenging assay

Scavenging effects of the extracts for DPPH radical was determined by the method of Yan and Chen (1995) with slight modifications. A solution of (0.15 mM) DPPH-EtOH was sonicated in hot bath until completely dissolved. Serial dilutions of the extracts were prepared in EtOH. The basic procedure was to add an aliquot (1 mL) of test sample to (1 mL) of DPPH -EtOH solution. The mixture was vortexed for 1 min and then incubated at room temperature for 30 min in the dark covered with aluminum foil. . The absorbance was read at 517 nm using a Jenway 6405 UV/Vis spectrophotometer, and the scavenging activity (%) (SA) was calculated as follows:

SA (%): $[1- (A_{sample} - A_{sample\ blank}) /A_{control}] \times 100$.

Sample solution (1 mL) plus EtOH (1 mL) was used as a sample blank, and DPPH solution (1 mL) plus EtOH (1 mL) was used as a negative control. Catechin and ascorbic acid were used as the positive controls(performed in triplicate). Stock solutions of catechin (0.8 mg/mL) and ascorbic acid (0.8 mg/mL) were diluted with EtOH to give concentrations ranging from 1.5 to 20 μg/mL. All measurements were performed in duplicate.

2.6 Statistical analysis

Descriptive statistics were performed in the present study; total phenol, flavonoids and terpenes contents of the 5 extracts using solvents with different polarities were summarized as mean. To examine the relationship between total phenol, flavonoids and terpenes contents and DPPH radical scavenging activity, Spearman's correlation coefficients were calculated between total phenol, flavonoids and terpenes contents and DPPH radical scavenging activity. Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 23. A p-value of < 0.05 was indicative of Statistical significance.

3. Results and discussion

3.1 Extraction yield, total phenol, total flavonoid, and total terpene contents.

In the recent years, edible flowers have gained popularity in cookbooks, visual media, and kitchen magazines. Edible flowers represent an important segment to expand food market. They have been used for centuries in many countries of the world not only for their aesthetic appearance, color, taste and shape, but also for their nutritional value; especially

that their nutritional profile meets several dietary demands including vegetarian/vegan (J. Takashi *et al.,*2020) and medical effect (F. Acikgoz, 2017).

Edible flowers have been used traditionally in cooking in various cultures' kitchens, such as (ancient Greeks, Romans, Middle East, Chinese and Indian cultures) (F. Acikgoz, 2017). They were used as: salad dressings, garnish, soups, desserts, and other food ingredients due to their valuable antioxidant contents, such as phenolic acid, flavonoids, and other phenolic compounds (Ngoitaku *et al.,* 2016). Phenolic compounds are associated with a high number of biological activities such as: the ability to break radical chain reactions, scavenge free radicals, and reduce the risk of cardiovascular diseases and cancer. And one with special interest is their antioxidant capacity that may help in protecting the cells against the oxidative damage ((Ngoitaku *et al.,* 2016, Felhi *et al.,* 2017). Due to this bioactive profile of edible flowers, food scientists gained attention in the development of functional foods (J. Takashi *et al.,*2020).

The yield of crude extracts from the edible flowers of *Cercis siliquastrum* obtained by maceration method using 5 type of solvents with different polarities (acetone, methanol, ethanol, dichloromethane (DCM), dichloromethane pretreated by NH_4OH (DCMa) were calculated and the results were shown in (Table 5). The recovery percentage of extractable compounds showed that dichloromethane-NH_4OH (DCMa) has the highest extraction yield

41% followed by ethanol 23%, methanol 21%, acetone 9%, and dichloromethane has the lowest extraction yield 7%.

Solvent extractions are most used procedures to extract polyphenol from plant materials due to their efficiency, ease of use, and wide applicability. Although, the extractability of a component depends on polarity, medium, pH, temperature, time of maceration, and the ratio of solute to solvent; however, the main recovery of phenolic compounds depends on the solubility of the phenolic compound, the type of the solvent and its polarity index (PI) (Felhi *et al.,* 2017). As reported by (Iloki *et al.,* 2015), polar phytochemicals are extracted in higher amounts in polar solvents and non-polar phytochemicals are extracted in higher amounts in non-polar solvents. And he reported also that the solubility of polyphenols depends mainly on the presence and position of hydroxyl groups and the molecular size and the length of constituent hydrocarbon chains.

Previous studies have been done on *Cercis siliquastrum*, where acetone and methanol had been used for determining their phytochemical composition and isolating their polyphenol content (S.Hattab *et al.,* 2019). In this study, the total phenolic (TPC), total flavonoid (TFC), and total terpenes content of *Cercis siliquastrum* were determined by Folin-ciocalteau, Aluminium Choloride and Salkowski test, using five different extracting solvents (acetone, dichloromethane pretreated by NH_4OH, dichloromethane, methanol and

ethanol) to increase the yield of extraction**;** and results were expressed in GAE/100mgDW, QE/100mg DW, and LE/100mg DW respectively.

For **the total phenolic content:** acetone solvent showed the highest extraction yield of phenolic compounds (4.825 mg GAE/100mg) followed by DCM (3.44 mg GAE/100mg), DCMa (3.35mg GAE/100mgDW), methanol (2.8mg GAE/100mg) and ethanol (2.65mg GAE/100mg) (Table6).

This difference in extraction appears to be related not only to the difference in the polarity of extracts of the components but also to the solvents used, in addition to the difference in structure of the phytochemical compounds which also plays a vital role in increasing the solubility of phytochemical compounds (Felhi *et al.,* 2016a). And related to the presence of other plant components such as glucose, fructose, sucrose, inorganic oil, amino acids, and vitamins (F. Acikgoz, 2017). Where all these chemical and nutritional composition varieties occur during flower stage of development, season, soil, climate, and other environmental factors (Ramalhosa *et al.,* 2019). Therefore, the selection of the solvent highly depends on the nature of the bioactive compound being used. Methanol and mathanoic solutions are the most frequent solvents used, however ethanol, acetone, isopropanol, water, ether, acetonitrile, and other solvents have been used also (Pires *et al.,* 2019, Ramalhosa *et al.,* 2017).

A study done by (Ivanka *et al.,* 2016) on *Calendula officinalis L.* and *Tagetes erecta L*. flowers, the highest yield of phenols was from the 80% methanol extracts.(Mohadjerani , 2012) used four different solvent {acetone, methanol, water, methanol: water(50:50, v/v)}, for the extraction of total phenolic content; results showed that the highest amounts were from methanol and aqueous methanolic extracts .Another study done by (Ammar *et al.,* 2015) on O.*ficus –indica* flowers using (water, acetone, dichloromethane, hexane, acetonitrile, acetone, and ethyl acetate) and their effect on the extraction yield were determined using Soxhlet extraction (SE) and maceration methods (ME). Results showed that the highest extraction yield was MeOH and water (highest polarity) for SE and ME respectively.

Also a study was done on the flowers and pericarp of immature edible fruit of *M.Oleifera,* where extraction was done by 50% ethanol and 70% acetone, results revealed higher values of phenolic in acetone extract in both parts of the plant (Siddhuraju *et al.,* 2014). Another study done by (Medini et al., 2014) on the shoot parts of *Limonium Deliccatulum* during vegetative and flowering season, using the following solvents (hexane, acetone/water (8;2, v/v), ethanol/water (9:1, v/v), methanol/water (8:2, v/v), and water. The results showed that in both stages, the maximum content was recorded in acetone extracts. (Sulaiman *et al.,* 2011) did a study on 37 species of vegetables using four different solvents (70%acetone,

70% ethanol, 70% methanol, and distilled water). 70% acetone was identified as the most efficient solvent for extracting polyphenolic antioxidants from the 24 out of 37 vegetables species, followed by methanol.

Another study was done by (Behebhe *et al.,* 2016) on black tea and herbal infusions from Zimbabwe and Brazil; using different solvents (hot water, 50 % methanol, ethanol, 50 % ethanol, acetone, 50 % acetone and ethyl acetate) to determine and compare the effect of several solvents on phenolic composition and free radical scavenging activity. For the black tea, *Camellia sinensis*, was used. Zimbabwean herbal infusions used were *Lippia javanica* and *Ficus sycamore*. While those from Brazil were *Syzygium jambolanum*, *Cuphea carthagenensis* and *Ilex paraguariensis*. Acetone (50 %) extracted a higher total phenolic content (TPC) in *C. sinensis*, *L. javanica* and *I. paraguariensis*. Aqueous organic solvents extracted higher quantities of phenolic compounds than in their absolute organic nature. Hot water extracted the highest TPC in *F. sycamore* and *S. jambolanum* while 50 % ethanol was highest in *C. carthagenensis*.

For **total flavonoid content**, ethanol showed the highest extractive capacity (53mg QE/100mg DW followed by acetone 51mg QE/100mg DW) , then DCM (49mg QE/100mg DW), where DCMa and methanol possessed the lowest extraction (34 and 26.5 mg QE/100mg DW) respectively (table 6). Epidemiological studies revealed that flavonoid-

rich diet exhibits multiple biological effects in addition to their antioxidant properties, such as: antibacterial, anti-viral, anti-inflammatory, vasodilatory, anti-ischemic, antioxidant, hepatoprotective, and anticancer. And the French Paradox showed that flavonoids increased longevity and reduced the incidence of cardiovascular diseases of French people despite the amount of fats they consume. Moreover, many studies showed that flavonoids can inhibit lipid peroxidation and platelet aggregation and improve increased capillary permeability and fragility. And in vitro antioxidant activity of flavonoid study, it showed that the antioxidant capacity of flavonoid is much stronger than those of vitamin A and C (D. Procházková *et al.,* 2011, Ling *et al.,* 2009. Yang *et al.,* 2015).

(Shi *et al.,* 2018), support this finding where the extraction of myricetin, quercetin, and afzalin from the leaves of C. *Chinensis* where dissolved in methanol, ethanol, water, and acetonitrile. The results showed that the three analytes (myricetin, quercetin, and afzelin) did not appear in the HPLC when dissolved in water and acetonitrile, and that ethanol was the best solvent to extract them. (Vongsak *et al.,* 2012) tried several extraction methods on the dried leaves of *M.oleifera L.* using 50 and 70% (v/v) ethanol as solvents, except for squeezing and decoction in which distilled water was used. Each method was done in triplicate. Results showed that 70% ethanol is the most suitable extraction method thus promoting the highest contents of total flavonoids. Another studied carried out by (Do *et*

al., 2013) on the roots of *Limnophila aromatic* using water and different concentrations of aqueous methanol, ethanol, and acetone (50%, 75%, and 100%).the results showed that the highest TFC was obtained in the 100% ethanol extract, followed by the 100% acetone extract, the 100% methanol extract, and the water extract. Another study done on the leaves of *Quisqualis Indica L.*by (Jasiem et al., 2018) using 70% ethanol and hexane for the extraction of different active compounds. The 70% ethanol extract included flavonoids, saponin, tannins and coumar, while hexane extract was containing only terpenes.

Another study done by (Munhoz *et al.,* 2014) on the extraction of flavonoids from flowers of *Tagetes patula* using different solvents: ethanol, acetone, and water. Acetone had the highest total flavonoid content, followed by ethanol.

For **total terpenes content,** dichloromethane showed to be the king of extracting terpene with capacity of (192 mg LE/100mg DW) dropped to less than half by acetone (75 mg LE/100mg DW) then DCMa with very low capacity (16.7 mg LE/100mg DW), whereas both methanol and ethanol showed very little extraction capacity towards terpenes (table 6). Terpenes are naturally occurring substances in both animals and plants; they belong to the large family of lipids. Monoterpenes and sesquiterpenes are major components of volatile oils that are abundantly found in fruits, flowers, and spices. Mankind has used terpenes that are extracted from plants for many different purposes such as fragrances and

flavors, as pharmaceutical agents: anticancer drug (Taxol), antimalarial drug (Artimesinin), antibacterial activity, antifungal, antiviral, and as insecticides (Gayathri *et al.,* 2014, Paduch *et al.,* 2007, Duarte-Almeida *et al.,* 2004). A study done by (Steinberg, 2017) on the bark of *C. canadensis*; the compounds identified through the extraction using dichloromethane were: monoterpenes (pinene, limonene, linalool) and pentacyclic triterpene (lupeol). Another study done by (Palma *et al.,* 2004) on the determination of terpenoids in wines by solid –phase (C-18 versus divinylbenzene-based), extraction and gas chromatography the eluting solvent (***n***-pentane, dichloromethane, ethanol and methanol). Highest recoveries were from dichloromethane (72%) obtained for all the solid phases. And thus, dichloromethane was selected as the best eluting solvent for terpenoids. (Yilmaz *et al.,* 2016) used dichloromethane and methanol solvent for the extraction of terpenoids from the aerial parts of *Nepeta obtusicrena.* A novel diterpenoid (obtusicrenone) and a novel triterpenoid (nemrutolone) were isolated from the dichloromethane extract of *N. obtusicrena* in addition to two known triterpenoids (oleanolic acid and ursolic acid).

3.2 Antioxidant activities of *Cercis siliquastrum* extracts using DPPH assay.

Interest in antioxidants is increasing every day; especially in those intended to prevent deleterious effects of free radicals in the human body. Therefore, there is a parallel increase in the use of methods for estimating the efficiency of these antioxidants (Sa ′ nchez-

Moreno, 2002; Schwarz, et l., 2001). One such method that is currently popular is based upon the use of diphenylpicrylhydrazyl (DPPH) because it is simple, inexpensive, low reagent and sample consumption, and high throughput analysis of antioxidant activity. In addition, DPPH is a stable organic radical with a characteristic absorption at 517nm; used to study the radical scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. Neutralizing free radical occurs when the antioxidants that are on interaction with DPPH either transfer an electron or a hydrogen atom to DPPH. Thus, the color changes from purple to yellow and absorbance decreases (Suárez-Jiménez et al., 2015).

The DPPH scavenging activity exhibited by *Cercis siliquastrum* extracts was expressed as IC_{50}% (table 7) defined as the equivalent concentration to give 50% effect or in other words 50% loss of DPPH activity. As, the lower the IC50, the higher the antioxidant activity of the extracts. These values were determined using the regression equations obtained for concentration activity curves (table 6). Our results showed that **acetone** extract has **highest** DPPH scavenging activity **41.85** mg/ml followed by **ethanol (71.68 mg/ml),** methanol (86.69 mg/ml) and DCMa (409.2 mg/ml), while DCM has the lowest activity 1274 mg/ml (table 7). Thus, acetone extract was found to be the most potent towards DPPH free radicals. Comparing our results with ascorbic acid the commonly used reference

compound, that is known to exhibit DPPH radical scavenging activity at an IC50 0.003 mg/mL, we noted that all extracts exhibited an activity lower than the reference group. Previous studies reported similar results; (1.75 mg/ml and 3.31 mg/ml), was exhibited by C. siliquastrum flowers acetone and methanolic fractions, respectively, followed by C. siliquastrum leaves methanolic and acetone fractions with IC50% (4.78 mg/ml and 8.31 mg/ml), respectively acetone was shown to have the highest antioxidant potential with IC50% (1.75mg/ml) in flowers of *Cercis siliquastrum* (J. Amer *et al.;* 2019). On the other hand, ethanol extract of the leaves of *Cercis chinensis* showed the highest antioxidant activity where IC50% (26.6 mg/ml) (M. Na *et al.;* 2010).

In another study done by (Na *et al.,* 2003), researchers reported that piceatannol IC50% (3mg/ml) derived from the leaves and stems of *Cercis chinensis* to have a potent radical scavenging activity.

3.3 Correlation between phytochemical constituents and antioxidant activity.

Correlation coefficients between the assessed phytochemical constituents and DPPH radical scavenging activity are reported in (table 8). Specifically, a weak negative correlation was found between TPC and IC_{50} DPPH scavenging activity (r -0.100, p 0.873), and a negative strong correlation was observed between TFC and IC_{50} DDPH scavenging

activity (r: -0.500, p: 0.391) which means that neither TPC nor TFC are responsible for the antioxidant activity. In addition, a positive moderate correlation was found between TTC and IC 50 DPPH scavenging activity (r: 0.359, p: 0.553). However, none of these relationships were found to be statistically significant.

In certain studies, it was reported that the quantity of phenolic compounds is positively correlated to the antioxidant activity, and in others it was reported that there is no positive relationship (Faujian *et al.,* 2009, Li *et al.,* 2009, Sun and Ho, 2005, Hesam *et al*., 2012, Rafat *et al.,* 2010). And in our study, there is no positive correlation between the phenolic compound and antioxidant activity.

Solvents	**Yield (mg)**	**% of Total Sum**
Acetone	0.51	9%
Dichloro+Ammonium	2.4	41%
Dichloromethane	0.39	7%
Methanol	1.23	21%
Ethanol	1.33	23%

Table 5: Extract yield means of *Cercis siliquastrum flowers* using 5 solvents with different polarities.

Table 6: Total phenol contents, total flavonoids content and total terpene content of the 5 extracts using solvents with different polarities in duplicates.

	Total phenol content		**Total flavonoid content**		**Total terpenes content**	
Solvents	Mean mgGAE/ 100mgDW *	%	Mean mgQE/ 100mg DW*	%	Mean Mg LE/100mg DW*	%
Acetone	4.82	28.3%	51	24%	75	26.4%
Dichloro+Amounnia	3.35	19.6%	34	16%	16.7	5.88%
Dichloromethane	3.44	20.1%	49	23%	192	67.7%
Methanol	2.80	16.4%	26.4	12.3%	NA	NA
Ethanol	2.65	15.5%	53	25%	NA	NA

Table 7. DPPH scavenging activity of 5 *C.siliquastrum flowers* extracts using solvents with varying polarities.

Solvent	**IC50% DPPH assay mg/ml**	**Regression equation**	**R^2**
Methanol	86.89	Y=0.574x+0.2381	0.9871
Ethanol	71.68	Y=0.6853x+0.8765	0.986
Acetone	41.85	Y=1.3204x-5.2596	0.9838
Dichloromethane	1274	Y=0.0423x-3.8879	0.9659
Dichloro+ammonium	409.2	Y=0.0887x+13.705	0.9842

Table 8: Correlation between total phenols, total flavonoids and total terpenes contents and antioxidant activities (DPPH radical scavenging).

	Correlation coefficient	**DPPH radical scavenging activity**
Total Phenol content	Correlation coefficient	-.100
	P value	.873
	N	5
Total Flavonoid content	Correlation coefficient	-.500
	P value	.391
	N	5

Total Terpenes content	Correlation coefficient	.359
	P value	.553
	N	5

4. Conclusion

Edible flowers are increasingly used by consumers and having special interest in food industry and culinary; not only for enhancing the visual appearance of various dishes but also- and majorly -for their nutritional characteristics. Such as low in fats content, high in energetic value, and natural source of secondary metabolites (phenols, flavonoids, and anthocyanins…) which play a major role in health promotion and disease prevention.

The screening of different extracts from the flowers of *Cercis siliquastrum* for total phenols, total flavonoids, total terpenes, and antioxidant activities was performed. Acetone was found to have the highest extraction capacity for total phenols, ethanol for total flavonoids, whereas; dichloromethane showed to show the highest extracting capacity for total terpenes content. Also, acetone exhibited the highest antioxidant against DPPH radical scavenging activities.

Strong positive correlation was found between total terpenes content and antioxidant activity which means that in this experiment; terpenes were the ones responsible for the antioxidant activity.

However, none of the correlations between antioxidant capacities and TPCs and TFCs was found to be statistically significant, this may be due to several reasons such as: small sample

size or due to the distribution of secondary metabolites which change during plant development that is: the content of polyphenols and flavonoids decrease during the flower development and terpenes content increase during maturation (O. Kaisoon *et al.,* 2011, A. Andre *et al.,* 2020).

The main strength of this study is that it is the first done on assessing the antioxidant activities of the flowers of *Cercis siliquastrum.* However, further research should be done in the future to aim which phytochemicals are responsible for the antioxidant activity taking into consideration the above-mentioned limitations.

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