Adel Yarizade et al /J. Pharm. Sci. & Res. Vol. 9(12), 2017, 2382-2387

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Investigation of Antiglycation and Antioxidant Potential of Some Antidiabetic Medicinal Plants

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**Abstract**

Non-enzymatic glycation of protein is the major cause of various diabetic complications. Medicinal plants having therapeutic potential against glycation are pivotal to treat diabetic complications. In this regard, with the aim of evaluation of antiglycation and antioxidant potential of four different plant extracts, 25 grams of fine powdered aerial part of *Oliveria decumbens* and *Citrullus colocynthis*, fruits of *Capparis Spinosa*, and seeds of *Ferula assa-foetida*, and *Vitex agnus-castus* and *Juglans regia* leaves were extracted in 100 ml methanol, ethanol, methanol-ethanol and water. *In vitro* antiglycation activity of extracts were examined by BSA-fluorescent assay and the DPPH free radical scavenging assay was used to evaluate antioxidant activity. Results clearly showed that all medicinal plants exhibited antiglycation activities but vary considerably from plant to plant and also between different solvents. Among all investigated medicinal plants, *V. agnus-castus*, *O. decumbens* and *C.colocynthis* exhibited better glycation inhibitory activity compared to other plants. *V. agnus-castus* showed best inhibitory activity as inhibition percent of all four its extracts was above 50% and among all extract of medicinal plants, methanolic extract of *V. agnus-castus* exhibited stronger antiglycation activity, so that glycated BSA was decreased about 77%. Also methanolic extract of *C. colocynthis* and methanolic and ethanolic extracts of *O. decumbens* significantly exhibited glycation inhibitory activity so that formation of glycated BSA was decreased 64.6%, 58.5%, and 58.75% respectively. Antiglycation activity of other medicinal plants assessed in this study was under 50% at all four different solvent. For instance about *J. regia* the best inhibitory activity was methanolic extract (46%), *C. spinosa* and *F.assa-foetida* ethanolic extracts and methanolic extracts exhibited 38.8% and 34% respectively. According to DPPH assay results, antioxidant potential of investigated medicinal plants (100µg/ml) based on mean scavenging activity are in the following order, *C. spinosa* > *F.assa-foetida* > *V.agnus-castus* > *O. decumbens* > *C. colocynthis* > *J.regia*. It can be concluded that *V.agnus-castus* with strong antiglycation activity and good DPPH scavenging activity has high therapeutic potential against glycation-associated diabetic complications.

**Keywords:** antiglycation, antioxidant, diabetes mellitus, diabetic complications, medicinal plant.

## INTRODUCTION

From ancient time medicinal plants had important role in human population to use as food and especially to treat various disease and injuries. According to the world health organization (WHO) about 80% of the population in developing countries use herbal medicines particularly in primarily health care due to economy, less side effects and easy availability. In addition to use plants as herbal remedy, medicinal plants are important source providing lead compounds for modern drug discovery. Drugs such as, metformin, taxol, reserpine, digoxin, morphine, digitoxin, vinblastine and vincristine are some important ones that derived from herbal plants that nowadays use to treat various disease such as diabetes, cancer, hypertension, atherosclerosis, depression, asthma, obesity and other chronic diseases [1].

Diabetes is one of the chronic diseases that affects people health and cause many complications in patients. According to the International Diabetes Federation (2015), over 400 million people are affected by diabetes worldwide, and 5 million deaths reported in 2015, every six seconds a person dies from diabetes, and also diabetes expenditure reached USD1.197 billion [2]. Nowadays, major medication for TDM treatment is using oral hyperglycemic drugs that usually have side effect in patients. For efficient use of herbal plants as remedy for diabetes and its complications, evaluation of their positive effects against diabetes complication is crucial. None-enzymatic glycation and oxidative stress are the most important factors involved in the complication of diabeties, hence, antiglycation and antioxidant are the most important features that herbal plants being evaluated for having this properties.

In diabetes mellitus, glucose forms covalent adducts with proteins through glycation. Glycation is non-enzymatic reaction between amino acid groups of protein and carbonyl group of reducing sugars that leads to formation of Advanced Glycation End Products (AEG) and thus clearly alter structure and function of proteins [3, 4] Glycation of various structural and functional proteins including collagen and plasma proteins (as fibrinogen, albumin and globulins) due to high blood glucose levels is the major cause of pathogenesis of diabetic complications such as neuropathy, nephropathy and retinopathy. In addition to diabetes, AGEs is observed in several important diseases such as end-stage kidney and heart diseases, Alzheimer´s disease, arthritis and ageing.

In addition to changing structure and function of proteins, Glycation have also deleterious effects on some other important molecules including nucleic acids and lipids that develop diabetic complications.

Studies showed that ROS are produced in diabetic conditions usually via the non-enzymatic glycosylation reaction, the electron transport chain in mitochondria and

membrane-bound NADPH oxidase that are activated under diabetic conditions, finally leads to oxidative stress and damage to cell components such as nucleic acids, lipids and proteins. So investigation of herbal plants especially blood glucose lowering plants with antioxidant and antiglycation activities is valuable for the treatment of diabetes and its complications. In this regard, six herbal plants chose to assess antiglycation and antioxidant properties. *V. agnus-castus* commonly known as Chaste tree or Chasteberry is widespread on riverbanks and on shores in the Mediterranean region, Southern Europe and in Central Asia [5]. *V. agnus-castus*, traditionally has been used as a folk medicine for the treatment of various ailments as digestive aid, sedative, anti-infective, acne treatment in teenagers, insect repellent, infertility improvement, menopause and relieving cyclic breast pain, [5-7]. Also in vivo studies demonstrated that extract of *V. agnus-castus* has good pharmaceutical properties including antihyperglycemic activity [6], pancreatic protective [7], memory and learning improvement [8], despite having this beneficial effects, no in vitro and in vivo experimental studies have been carried out to assess the antiglycation property of *V. agnus castus*. Phytochemical analysis of *V. agnus castus* revealed that the fruits, flowers and, leaves of this plant contain flavonoid, iridoids, diterpenoids, volatile oils, and tannins [9]. *C. colocynthis* also known as bitter apple belong to Cucurbitaceae, widely distributed in the desert areas of the world. *C. colocynthis* fruits have wide range of medicinal uses in traditional medicine including diabetes, constipation, asthma, bronchitis, jaundice, joint pain and cancer. [10]. Antioxidant activities and therapeutic potential of this medicinal plant against diabeties mellitus, have been previously demonstrated in many studies [11-13]. Oral administration of 300 and 500 mg/kg doses of *C. colocynthis* fruit petroleum ether extract exhibited a significant reduction in blood glucose level in diabetic rats. Phytochemical investigation showed that major chemical constituents of the extracts were alkaloids, glycosides, terpenes and saponins. [11]. Investigation of different extract of *C. colocynthis* revealed that acetate, hydromethanolic and aqueous extracts exhibited significant antioxidant properties with IC50 350, 580 and 500 respectively [13]. The 200 and 250 mg/kg bw, saponosides crude extract isolated from the seeds of *C. colocynthis* L., significantly decrease the blood glucose level 48 h after administration to STZ diabetic rats [12]. Aqueous and acetone extracts from different parts of *C. colocynthis* were investigated by Marzouk et al (2010) for their scavenging capacity on 2, 2-diphenyl-1- picrylhydrazyl (DPPH) and their radical ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] cation scavenging activity. Results showed that all extracts exhibited a dose-depending antioxidant activity. They concluded that results strongly support the use of *C. colocynthis* as an important source of natural antioxidant agents [14].

*C. spinosa* is a perennial spiny shrub growing in hot and dry or arid/waterless climate. This plant is used in traditional medicine as a diuretic, treatment of gout, arthritis, rheumatoid, paralysis and neurological conditions as well as liver disorders [15]. In recent years, several biological activities of *C. spinosa* including antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and anticancer effects were reported in experimental and clinical studies. Taghavi and his coworkers reported that changes in liver, pancreas and kidney of diabetic rats treated with *C. spinosa* fruit extract, are very low compare to considerable changes in untreated diabetic rats. They mentioned that decrease in level of liver enzymes, creatinine, and other factors supporting the protective effects of *C. spinosa* fruit extract [15].
*O. decumbens* belongs to Apiaceae (Umbelliferae) family and is an endemic plant to Iran that grows in high temperature areas of south and west of Iran. In traditional medicine, it is used for indigestion, diarrhea, abdominal pain and fever [16] and has strong antimicrobial activities against a board spectrum of bacteria and fungi such as *Staphylococcus aureus* [17].

*F. assa-foetida* L. is a perennial herbaceous plant belongs to the Apiaceae family that grows in central Asia, Iran to Afghanistan, from which it is exported to the entire world. In folk medicine, it is used for the treatment of weak digestion, stomach ache, flatulence, asthma, epilepsy, intestinal parasites and influenza [18, 19]. Recent pharmacological and biological studies have reported several activities, such as antidiabetic effect [19], antioxidant [20], antimicrobial [21], antiviral [22], antispasmodic and hypotensive [23].

*J. regia* L. (Juglandaceae) has been widely used in Iranian folk medicine as a remedy for diabeties because of its blood glucose lowering activity [24]. Therapeutic potential of different parts of *j. regia* L. such as kernel, leaves and septum in the treatment of diabetes confirmed in many studies [25-27]. Oral administration of 250 mg/kg cyclohexane extract of walnut leaf significantly decreased blood glucose in the diabetic rats [25]. In alloxan-induced diabetic rats treated with ethanolic extracts of *J. regia*, insulin level increased and fasting blood glucose decreased and glycosylated hemoglobin decreased significantly [26].

In addition to hypoglycemic activity, there is a significant interest in identifying therapeutic potential of herbs against diabetic complication. In this regard, this study was conducted to evaluate therapeutic potential of the mentioned medicinal plant against diabetic complications via antiglycation and antioxidant assays.

## MATERIAL AND METHODS

### Sample preparation

Briefly, the aerial part of *O. decumbens* and *C. colocynthis*, fruits of *C. Spinosa*, and seeds of *F. assa-foetida*, and *V. agnus-castus*, and of *J. regia* leaves were collected, shade dried and then powdered using grinder. For methanol, ethanol, methanol-ethanol and water fraction, 25 grams of fine powdered tissues were soaked in 50 ml mentioned solvents on an orbital shaker for 24 hours. Then, plant debris was removed from resulting extracts using Whatman No. 1 filter paper and the filtrate was allowed to dry at

room temperature. Dried extracts were weighed and dissolved in dimethyl sulfoxide (DMSO) to yield a stock solution. The solution was stored at 4°C for subsequent use.

**Antiglycation assay**

*In vitro* antiglycation activity of the selected herbal plants were examined by BSA-fluorescent assay according to Choudhary and his coworkers with slight modification [28]. First bovine serum albumin (BSA), Glucose and sodium azide were dissolved in 100 mM phosphate buffer (pH 7.4). Then 20 µL BSA (10 mg/mL), 20 µL of glucose anhydrous (50 mg/mL), and 20 µL test sample (extract) was added to each well of 96-well plate assay and finally 20 uL sodium azide (14 mM) was added to inhibit bacterial growth. A well contained 20 µL glucose, 20 µL BSA, 20 µL sodium phosphate buffer (0.1 M, pH 7.4) used as control and 20 µL BSA and 40 µL sodium phosphate buffer used as blank. Reaction mixture was incubated at 37 $^0$C for 9 days and then 60 µL TCA (100 %) was added in each well and centrifuged (15,000 rpm) for 4 minutes. Supernatant discarded and the pellet was washed with 60 µL 5 % TCA and pellet was dissolved in 60 µL PBS.

AGEs formation was measured (excitation at 370 nm and emission at 440 nm) using BioTek™ Cytation™ 3 Cell Imaging Multi-Mode Reader (BioTek, USA).

The results are reported as follows: % Inhibition = [1 - (Absorbance extract/Absorbance control)] x 100.

**Antioxidant assay**

In order to measure antioxidant activity of plants extracts, the DPPH free radical scavenging assay was determined spectrophotometrically according to Yang et al [29]. For each sample, extract solutions were prepared by dissolving 100 µg of dry extracts in 1 ml of methanol or 10% DMSO. The reaction mixtures in the 96-well plates consisted of sample (100 µL) and DPPH radical (100 µL, 0.2 mM) dissolved in methanol. The mixture was stirred and left to stand for 15 min in dark. Then the absorbance was measured at 517 nm against a blank. All determinations were performed in triplicates. The radical scavenging activity was calculated as a percentage of DPPH decolorization compared to control. The radical-scavenging activity was expressed as percentage of DPPH decolorization and calculated using flowing equation:

% scavenging activity = [1 - (Absorbance extract/Absorbance control)] x 100.

**Statistical Analysis**

All experimental results were presented as means ± SD in triplicate. The statistical analyses were performed by one-way ANOVA. P value < 0.05 was regarded as significant. All statistical analyses were performed using SPSS software.

## RESULT AND DISCUSSION

Screening of traditionally used medicinal plants regarding to antiglycation properties would be beneficial in treatment of various disease complications specially, diabetes mellitus. Advanced glycation endproducts (AGEs) play an important role in the pathogenesis of major diabetic complications such as nephropathy and vascular disease. Modifications can clearly alter structure, enzymatic activity and biological half-life of proteins, cause mutations in DNA, and affect transport and signaling processes by damages to membrane lipids [30]. Studies demonstrated that some medicinal plants have ability to inhibit the process of protein glycation, AGEs formation and thus preventing alteration of the biological activity of protein. Results obtained from this study clearly showed that all investigated medicinal plants exhibited antiglycation activities but vary considerably from plant to plant and also between different solvents (fig.1). Among all investigated medicinal plants, *V. agnus-castus*, *O. decumbens* and *C.colocynthis* exhibited better glycation inhibitory activity compared to other plants. *V. agnus-castus* showed best inhibitory activity as inhibition percent of all four its extracts was above 50% and among all extract of medicinal plants, methanolic extract of *V. agnus-castus* exhibited stronger antiglycation activity, so that glycated BSA was decreased about 77% (fig.1). Also methanolic extract of *C. colocynthis* and methanolic and ethanolic extract of *O. decumbens* significantly exhibited glycation inhibitory activity so that formation of glycated BSA was decreased 64.6%, 58.5%, and 58.75% respectively. Antiglycation activity of other medicinal plants assessed in this study was under 50% at all four different extracts. For instance about *J. regia* the best inhibitory activity was methanolic extract (46%), for *C. spinosa* and *F.assa-foetida* ethanolic extract and methanolic extract exhibited 38.8% and 34% respectively.

According to high antiglycation activity of *V. agnus-castus*, it is rational to infer that *V. agnus-castus* has valuable therapeutic potential in preventing and treatment of diabetic complications. In addition to traditionally use of *V. agnus-castus* as a folk medicine in various ailments, the pharmaceutical potential of *V. agnus-castus* is demonstrated in many studies such as, antihyperglycemic activity [6], pancreatic protective [7], memory and learning improvement [8], and anti-cancer activity [31]. Stella and his coworkers when used methanolic extract of *v. agnus-castus* to assess antihyperglycemic activities observed that 50, 100 and 200 mg/kg significantly increase serum insulin and decrease blood glucose levels in STZ diabetic rats (Stella et al. 2011). Results obtained from this study is the first report of antiglycation properties of *V. agnus-castus* against AEG formation.

These fractions were then evaluated for their scavenging potential against DPPH. It has been reported that oxidative stress contribute to the formation of some AGEs, at same time, formation of AGEs leads to formation of reactive oxygen species [32]. Thus, in oxidative stress, cell component damaged and AGE formation intensify oxidative stress and complication of some diseases such as diabeties. So in case of using medicinal plant in complementary medicine, finding a plant with both antiglycation and antioxidant properties, will be an important step to treat or prevent disease complication. In this study, *V.agnus-castus* exhibited proper antioxidant activity (fig-1) that is in line with previous studies. The aqueous and ethanolic extracts of leaves and fruits showed

strong antioxidant activity [9]. Also methanolic extract of leaves and fruits of *V.agnus-castus* investigated by Gökbulut et al showed that both leaf and fruit methanolic extracts exhibited significant radical scavenging activity with IC50 values of 0.449±0.001 mg/mL and 0.612±0.004 mg/mL, respectively [33]. Makhmoor and his colleagues isolated nine compound including artemetin, casticin, 3-hydroxy-5,6,7,4 -tetramethoxy flavone U, penduletin, p-hydroxybenzoic acid, methyl 3,4-dihydroxybenzoate, methyl isovanillate, vanillic acid and 3,4-dihydroxybenzoic acid from, *V.agnus-castus* that methyl 3,4-dihydroxybenzoate and 3,4-dihydroxybenzoic acid which are derivatives of benzoic acid, exhibited significant scavenging activity against the DPPH free radical [34].

According to DPPH assay results, antioxidant potential of investigated medicinal plants (100µg/ml) based on mean scavenging activity are in the following order, *C. spinosa* > *F.assa-foetida* > *V.agnus-castus* > *O. decumbens* > *C. colocynthis* > *J.regia*. As mentioned above, *C. spinosa* showed strongest antioxidant activity than other medicinal plants, which it can be explained with its chemical content such as flavonoids, phenols, etc. So chemical investigation of most active fractions of medicinal plants to identify the main bioactive compounds that play a major role in antioxidant activity is interesting for researcher. Yang and colleagues reported that ethyl acetate fraction of ethanolic extraction of *C. spinosa* fruits that had good antioxidant activities (SC50 values of 0.321 mg dried raw material equivalents/mL, ) contains seven compounds that have good antioxidant activity including cappariside (SC50= 0.204 ± 0.002 mM), protocatechuic aldehyde (0.007 ± 0.0 mM), ethyl 3-4-dihydroxybenzoate (0.011 ± 0.0 mM), syringic acid (0.044 ± 0.002 mM), protocatechuic acid (0.032 ± 0.0 mM), vanillic acid (0.09 ± 0.001 mM), and 4-hydroxybenzoic acid (0.35 ± 0.017 mM), and five known compound that exhibited poor antioxidant activity including, 5-hydroxymethylfurfural, 5-hydroxymethyl furoic acid, 2-furoic acid, succinic acid, E-butenedioic acid [35].

Polyphenols and flavonoids are two important compounds that their correlation with antioxidant activities reported in many studies. Spectroscopic analysis of aqueous extract of *C. spinosa* fruits led to the identification of 13 compounds that flavonoids, indoles, and phenolic acids are the major of them [36]. According to Allaith (2016) antioxidant capacity in *C. spinosa* fruits strongly correlated with the total free phenolics and total flavonoids. Fractionation of *C. spinosa* fruits methanolic extract revealed that hydrophilic fraction has higher antioxidant activity that is an indicative that the major contributors of the antioxidant activity in caper fruits are water soluble constituents or the phenolic compounds. Also methanolic seed extract that had higher total free phenolics and total flavonoid contents exhibited remarkably higher antioxidant activity, which indicate that higher antioxidant activity found in seeds may be attributed to the presence of phenolic compounds [37]. Correlation between antioxidant activity and phenolic and flavonoid content, also, have been reported by Bhoyar and coworkers [38], as maximum and minimum DPPH and ABTS radical scavenging activity was observed in leaves that contain highest phenolic and flavonoid content and leaves that contain lowest phenolic and flavonoid content, respectively. This study revealed that the fruits of *V.agnus* castus showed strong antiglycation activity and *C. spinosa*, as determined by DPPH assay, had higher antioxidant activity than other investigated medicinal plants. Thus, fruits of *V.agnus* castus and *C. spinosa* can be used as a potential source of natural antiglycation and antioxidant substance and also as a remedy for diabetic complications. However, further studies on the antiglycation components of *V.agnus castus* fruit extracts and *in vivo* evidence are required.

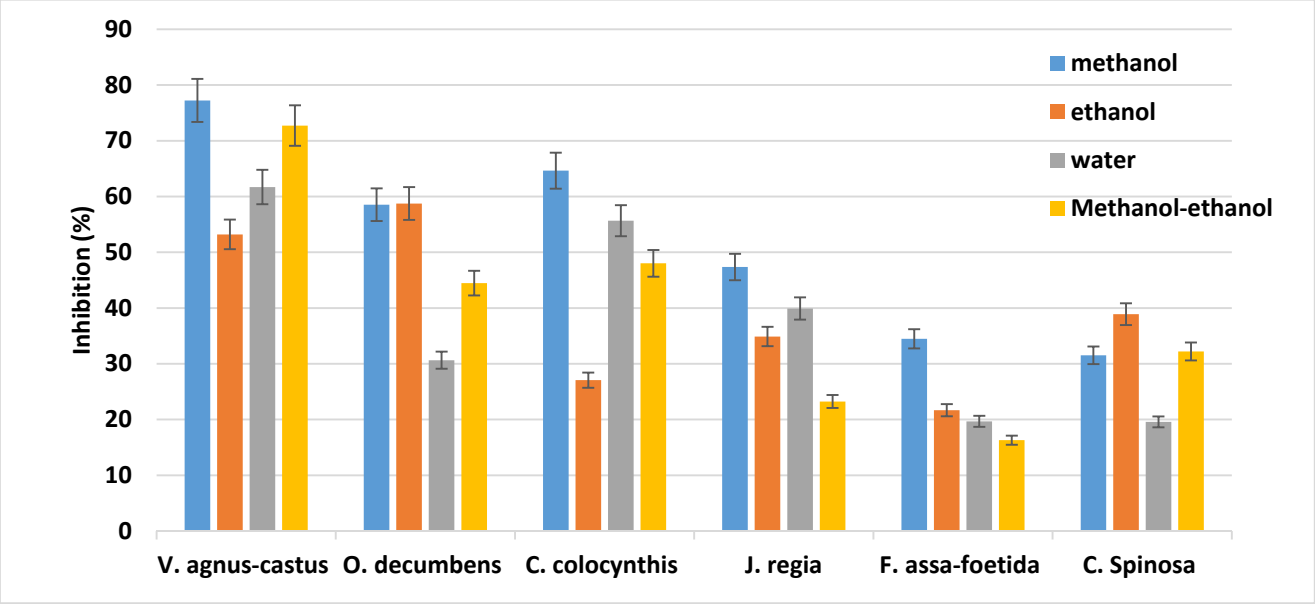


Figure 1. Inhibitory activity of different extracts of evaluated medicinal plants

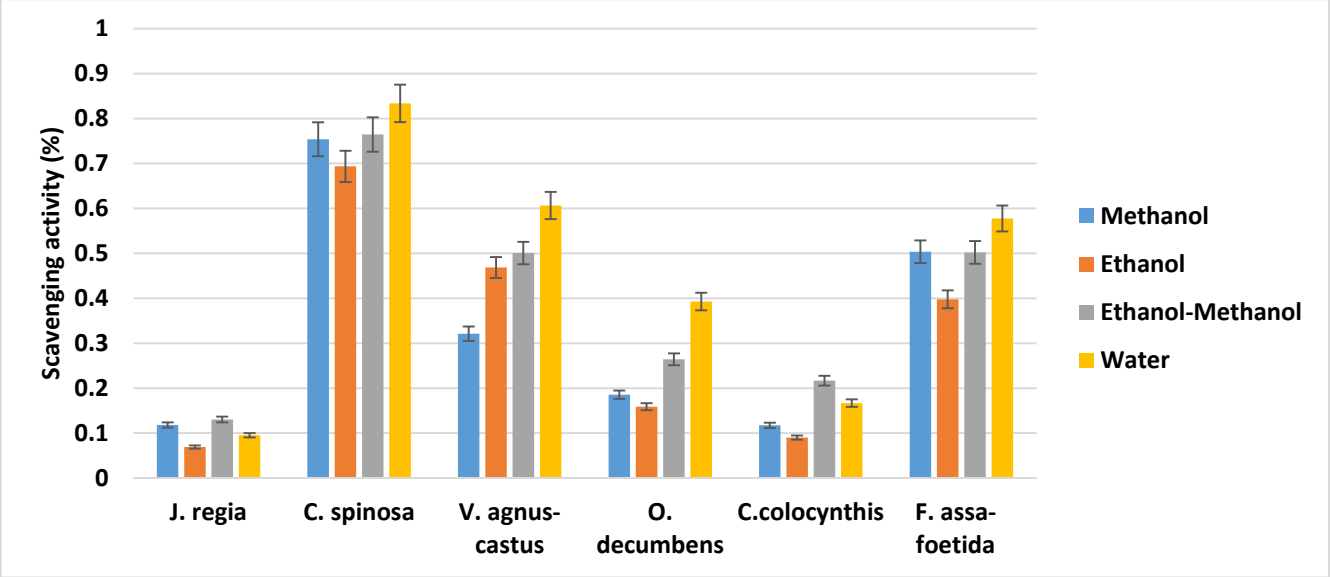


Figure 2- Scavenging activity of investigated medicinal plants on DPPH radicals.

## CONCLUSION

AGEs are produced under oxidative stress in various diseases especially diabetes mellitus and it is a major cause in the development of the various diseases complications. Nowadays, medicinal plants are an attractive target as natural resources to identify effective lead compounds in diabetic complication treatment. In this study evaluation of the antiglycation and antioxidant properties of six medicinal plant clearly showed that most of them have significant therapeutic potential against diabetic complication but *V.agnus castus* showed strongest antiglycation inhibitory activity that can be concluded that this medicinal plant might provide effective lead compound for treatment against the glycation reaction and oxidative stress in diabetic patients.

## ACKNOWLEDGMENTS

The present research was conducted in Shiraz University and financially supported by Ministry of Science, Research and Technology of the Islamic Republic of Iran.

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