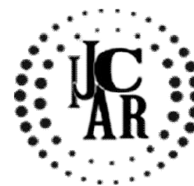
International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 01 (D); January 2020; Page No.21060-21065
DOI: http://dx.doi.org/10.24327/ijcar.2020.21065.4130



Research Article

COMPARISON OF DIFFERENT MODIFICATIONS OF DPPH METHOD FOR THE ESTIMATION OF RADICAL SCAVENGING ACTIVITY OF *SILYBUM MARIANUM (L.)*

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A R T I C L E   I N F O

Article History:
Received 6th October, 2019
Received in revised form 15th November, 2019
Accepted 12th December, 2019
Published online 28th January, 2020

Key words:

Silybum marianum, Silymarin, flavonolignans, DPPH, radical scavenging activity, plant extracts.

A B S T R A C T

Silybum *marianum (L.)* is a medicinal plant containing Silymarin – a complex of flavonolignans: Silybin A, Silybin B, Isosilybin A, Isosilybin B, Silychristin A, Silychristin B, Silydianin, Silandrin, Isosilandrin, Silychermin, Neosilychermin, Siliamandine and Siliymonine. Silymarin possesses antioxidant, anticarcinogenic, anti-inflammatory, antifibrotic, hepatoprotective, immunomodulatory and neuroprotective activity and is applied in liver cirrhosis, acute and chronic viral hepatitis. Hepatoprotective action is a result of antioxidant, anti-lipid peroxidative activity and of reduction of glutathione oxidation.

Objective: The aim of cuttent study was to compare the different modificated DPPH methods for determination of free radical scavenging activity of *Silybum marianum L.* extracts.

Methods: A literature survey has been applied by using of different data bases as Scopus and Medline, Google Scholar, PubMed.

Results:

For the estimation of free radical scavenging activity of *Silybum marianum L.* extract, the modifications of DPPH method comprise the changing of :

1) reaction time (from 30 min. to 90 min.)
2) solvent for DPPH (methanol, ethanol, butanol)
3) concentration of DPPH (from 0.1 M to 0.006 mM, 0.15 mM, 0.2 mM, 0.3 mM, 1 mM)
4) proportion between volumes of DPPH and plant extracts
5) absorption maximum: from λ = 517 nm to λ = 515 nm or λ = 520 nm.

Conclusion:

For different modifications of DPPH method for the assessment of free radical scavenging activity of *Silybum marianum L.* extract, the following most important conclusions can be summarized:

I) the most often applied modifications are in connection with the changing of:
1) concentration of DPPH solution: 0.1 mM to 0.006 mM, 0.15 mM, 0.2 mM, 0.3 mM, 1 mM,
2) proportion between volumes of DPPH and plant extract

II) the most often used conditions for DPPH method are:
1) 30 min. reaction time between DPPH solution and plant extracts
2) application of methanol as solvent
3) absorption maximum: λ = 517 nm.

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INTRODUCTION

Chemical compounds in Silybum marianum L.

Silybum *marianum (L.) Gaertn.* (milk thistle, Marian thistle, Mediterranean milk thistle). (Asteraceae) is an important medicinal plant both in Europe and Asia. The crude commercial product, termed Silymarin is a complex of at least 7 flavonolignans and comprises 65 % to 80 % of milk thistle extract: Silybin A (Silibinin A), Silybin B (Silibinin B), Isosilybin A, Isosilybin B, Silychristin A, Silychristin B, Silydianin. The term "Silymarin" have been introduced in 1968 by the German phytochemist prof. Hildebert Wagner. From Silymarin has been derived a semipurified fraction – Silibinin: 1 : 1 mixture of 2 diastereoisomers: Silybin A and Silybin B. A similar diastereoisomeric mixture contains Isosilybin A and Isosilybin B, which are regioisomers of Silybin A and Silybin B (Fig. 1.). The other flavonolignans are Silychristin A, Silychristin B and Silydianin (Fig. 1.) [1]. Other new found flavonolignans are Silandrin, Isosilandrin, Silychermin, Neosilychermin [2], Siliamandine and Siliymonine (Fig. 2.) [3]. Silibinin is found in the roots [4] while Silyamandin can be found in the fruits [3].

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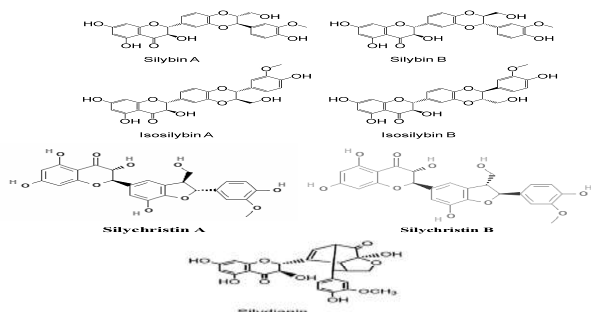

Fig 1 Chemical structures of Silibin and Isosilybin, Silychristin and Silydianin.

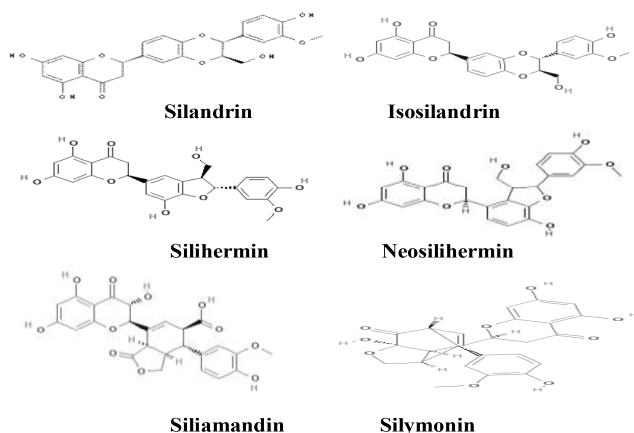

Fig 2 Chemical structures of Silandrin, Isosilandrin, Silihermin, Neosilihermin, Siliamandin and Silymonin.

Silymarin possesses hepatoprotective [5, 6], antioxidant [6, 7], anticarcinogenic, immunomodulatory [8], anti-inflammatory, antifibrotic [9], neuroprotective and neurotropic activity [8]. Flavanolignans from the seeds of *Silybum marianum L.* are allosteric protein tyrosine phosphatase (PTP) 1B inhibitors [10] and their effectiveness in ameliorating diabetes in-vitro or in-vivo has been reported. The increased insulin sensitivity and improved glucose tolerance are results from the inhibition of PTP 1B [11, 12].

In oxidative stress, the reactive oxygen species induce peroxidation of polyunsaturated fatty acids in the cell membrane bilayer, which causes oxidation of lipids, proteins, DNA and RNA, and damaging of other cellular components [9]. Free radicals play an important role in the pathogenesis of cardiovascular diseases; atherosclerosis [13]; neurodegenerative disorders [14] as Alzheimer's [15] and Parkinson's disease; alcohol induced liver disease [16]; cancer and psoriatic arthritis [17].

Natural antioxidants present in food of plant origin protect against free radicals and are important for good health [18, 19]. Silymarin and it's main isomer Silibinin (Silibin) have been shown to possess antioxidant properties. By DPPH method has been proved higher activity (78.2 %) of seeds of the purple flowering plant than seeds of white flowering plant (49 %). For white flowering plant the activity is 64.8 % for young leaves and 67.2 % for roots, which is higher than the purple flowering plant: 55.1 % activity for young leaves and 65 % for roots. The maximum antioxidant activity of leaves have been observed from 80-day-old plants [20]. Extract from the cultured cells of *Silibum marianum L.* show 48 % inhibition, compared to 55 % inhibition of the extract from the fruits [21].

Silymarin is applied in liver cirrhosis, acute and chronic viral hepatitis, toxin or drug-induced hepatitis. Silymarin and Slibinin hepatoprotective action is a result of antioxidant, anti-lipid peroxidative and free radical scavenging actions, reduction of glutathione oxidation and stimulation of hepatocyte regeneration by induction of protein synthesis. Silymarin possesses anti-inflammatory and anticarcinogenic properties by inhibition of the transcription factor NF-kB, which regulates the expression of genes involved in the inflammatory process and carcinogenesis. Anti-inflammatory action is results of inhibition of leukotriene synthesis and formation of prostaglandins [9]. Silibinin inhibits proliferation and promotes cell-cycle arrest of human colon cancer [22].

In-vivo and in-vitro methods for evaluation of antioxidant activity

In-vivo methods for evaluation of antioxidant activity include: Catalase (CAT) method: λ = 240 nm; Glutathione peroxidase (GSHPx) estimation: λ = 340 nm; Glutathione-S-transferase (GST) assay: λ = 340 nm; γ-Glutamyl transpeptidase activity (GGT) assay: λ = 405 nm; Reduced glutathione (GSH) estimation: λ = 412 nm; Superoxide anion scavenging activity assay (SOD) method: λ = 420 nm; Lipid peroxidation inhibitory activity (LPIA) assay: λ = 532 nm; Low Density Lipoprotein (LDL) assay: λ = 532 nm; Ferric reducing ability: using FRAP reagent: TPTZ (2,4,6-tripyridyl-s-triazine) and ferrochloride: λ = 593 nm; Glutathione reductase (GR) assay [23-25].

In vitro methods for evaluation of antioxidant activity are: Hydrogen peroxide scavenging activity (HPSA) assay: λ = 230 nm; Xanthine oxidase method: λ = 293 nm**;** β-carotene linoleic acid method/conjugated diene assay: λ = 470 nm; Peroxynitrite radical scavenging activity: λ = 485 nm; Ferric thiocyanate method (FTC) method: λ = 500 nm; N,N-dimethyl-p-phenylene diamine dihydrochloride (DMPD) method: λ = 505 nm; 1,1-diphenyl-2-picryl-hydrazyl radical scavenging activity (DPPH) method: λ = 517 nm; Oxygen radical absorbance capacity (ORAC) method: λ = 520 nm**;** Hydroxyl radical scavenging activity assay (HRSA) assay: λ = 532 nm; Nitric oxide scavenging activity: λ = 546 nm; Thiobarbituric acid (TBA) method; λ = 552 nm; Superoxide radical scavenging activity (SOD): λ = 560 nm**;** Metal chelating activity: λ = 562 nm; Ferric reducing-antioxidant power (FRAP) assay: λ = 593 nm; Phosphomolybdenum method: λ = 695 nm; Reducing power method (RP): λ = 700 nm; 2,2'-azino-bis3-ethylbenzthiazoline-6-sulfonic radical cation decolourisation assay (ABTS): λ = 750 nm; Total radical-trapping antioxidant (TRAP) method; Hydroxyl radical scavenging activity (HRSA) assay [23-27].

DPPH radical scavenging activity

DPPH antiradical activity assay is based on the reduction of a stable free radical DPPH (α,α-diphenyl-β-picryl-hydrazyl). The delocalization of electron gives rise to the deep violet color, characterized by an absorption at λ = 517 nm [28] or at λ = 520 nm [29]. As electron becomes paired off in the presence of a hydrogen donor, a free radical scavenging compound, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured. The substrate, that can donate a hydrogen atom, reduce DPPH to DPPHH, the absorbance

decrease and the violet DPPH is transferred to yellow (1,1′-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine (DPPHH) (Fig. 3.) [29].

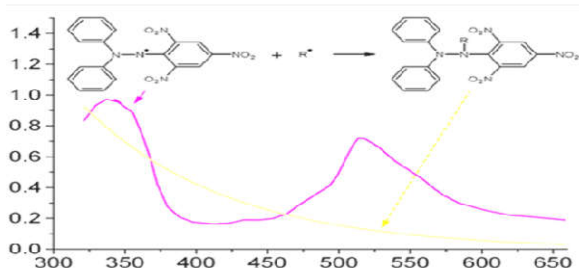

Fig 3 Reaction of compound with DPPH (violet) to 1,1′-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine (yellow).

In accordance to Blois for DPPH method, 1 ml 0.1 mM DPPH solution in methanol is added to 3 ml of various concentrations of methanol extracts and reference standard Butulhydroxytoluene (BHT) (125 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml) and after 30 min. at 25 ºC in dark the absorbance is measured at λ = 517 nm [30].

For the assaying of free radical scavenging activity of methanolic extract of medicinal plants, different modifications of DPPH method of Blios has been applied by changing of:

1. temperature (from 25 ºC to 27 ºC)
2. reaction time (from 30 min. to 20 min. or 100 min.)
3. concentration of DPPH solution (from 0.1 mM to 0.2 mM, 0.3 mM, 0.4 mM)
4. proportion between volumes of DPPH methanol solution and extracts
5. solvent for DPPH (from methanol to ethanol).

In all of these methods compounds reacts with DPPH, which is a stable free radical, that is reduced to the DPPHH and as consequence the absorbance decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the compounds in extracts through hydrogen donating ability [28].

For the assaying of free radical scavenging activity of methanolic leaf extract of medicinal plant *Cryptolepis grandiflora*, the modification of DPPH method of Blios has been used: 5 ml 0.1 mM DPPH radical solution and 5 ml methanolic leaf extract of plant have been incubated for 20 min. at 27 ºC in dark after that the absorbance have been recorded at λ = 517 nm [31].

Kumar *et al*. have been determined the scavenextract of *Hydrocotyle conferta* on DPPH radicals according to the method of Shimada *et al*. [32]: 1 mging effect of methanolic l 0.2 mM methanolic solution of DPPH have been mixed with 4 ml of various concentrations of extracts and after 30 min. at 25 ºC in dark, the absorbance has been measured at λ = 517 nm [33].The hydrogen donating ability of *Vernonia cinerea* has been examined by modification of DPPH method of Blios, by mixing of 1 ml 0.3 mM DPPH methanol solution and 1 ml extract of *Vernonia cinerea* and after 30 min. reaction at room temperature the absorbance values have been measured at λ = 517 nm using methanol as blank. Ascorbic acid has been taken as the positive control and 1.0 ml 0.3 mM DPPH solution with 1 ml methanol has been served as negative control. The scavenging activity against DPPH (% inhibition) free radical has been calculated by using: absorbance of the control ($A_{control}$) and absorbance in the presence of the extract (A_{test}) by the following equation: % inhibition = $[(A_{control} - A_{test}) \cdot 100] / A_{control}$ [34].

The scavenging effect of *Costus pictus* leaf extraxts [35] and of coumarin compounds [36] has been determined by the application of the modification of DPPH method of Blios: reaction between 1 ml 0.3 mM DPPH methanol solution and 1 ml extracts, and after 30 min. at 25 ºC in dark, recording the absorbance at λ = 517 nm.

In modification of DPPH method from Brand-Williams, the reaction mixture consists of 0.5 ml sample, 3 ml absolute ethanol and 0.3 ml 0.4 mM DPPH solution in ethanol and the control solution contains 3.5 ml ethanol and 0.3 ml DPPH solution. After 100 min. incubation, the following absorbances: of sample (Abs_{sample}), control solution ($Abs_{scontrol}$) and compensatory solution (Abs_{blank}), has been detected) at λ = 517 nm, by using as compensatory solution a mixture of 3.3 ml ethanol and 0.5 ml sample. Antiradical activity: AA (%) is calculated by the following equation [37]:

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})x100}{Abs_{control}} \right]$$

Ekezie *et al*. have been analysed antioxidant potential of *Momordica charantia* by reaction of 5 ml 0.004 % DPPH solution with 5 ml of plant extract for 30 min. at 25 ºC in dark and the absorbance has been determined at λ = 517 nm [38]. For the evaluation of antioxidant potential of tomato powder, after reaction of 5.0 ml 0.04 % DPPH solution and 1 ml extract for 30 min. at 25 ºC in dark, the absorbance has been measured at λ = 517 nm [28].

The very often applied method for determination of radical scavenging activity of *Silybum marianum L.* extracts against DPPH includes incubation of DPPH solution with extract for 30 min. at room temperature 25 ºC in dark and measuring of the absorbance at λ = 517 nm.

For the assessment of free radical scavenging activity of Silymarin, the method of Blois, with the following modifications has been reported: a mixture from 0.5 ml 0.1 mM DPPH ethanol solution and 1.5 ml of Silymarin solution in ethanol at different concentrations (10 mg/ml – 30 mg/ml) has been incubated for 30 min. at 25 ºC in dark and the absorbance has been measured at λ = 517 nm [39].

DPPH radical scavenging activity of *Silybum marianum L.* seeds has been studied by application of DPPH method of Blois: reaction between 1 ml 0.1 mM DPPH methanol solution and 3 ml of *Silybum marianum L.* seed ethanol extracts with different concentrations (5 μg/ml, 10 μg/ml, 25 μg/ml, 50

μg/ml, 100 μg/ml and 150 μg/ml). The absorbance has been measured at λ = 517 nm after incubation in for 30 min at 25 ºC in dark [40].

For the estimation of free radical scavenging activity of *Silybum marianum L.* extracts, the very often modifications of DPPH method of Blios include the changing of solvent for DPPH (from methanol to ethanol) and the concentration of DPPH (from 0.1 mM to 0.006 mM, 0.15 mM, 0.2 mM, 1 mM, 0.002 %, 0.004 %). The following methods reported, illustrate these changes.

The DPPH radical scavenging activity of the seed extracts of wild *Silybum marianum L.* populations growing in Greece, has been determined using a method proposed by Blois, with some modifications: 3 ml 0.06 mM DPPH methanol solution has been reacted with 200 μl of seed extract, or 200 μl 1.0 m M Ascorbic acid standard or 200 μl methanol (control) for 30 min. at 25 ºC in dark and the absorbance has been detected at λ = 517 mn [41].

The inhibitory effect of different concentrations of Silymarin (0.05 mg/ml – 2 mg/ml) on DPPH have been estimated by measuring of the absorbance of the reaction mixture of 1.95 ml 0.1 mM DPPH ethanol solution and 0.05 ml Silymarin at λ = 517 nm after incubation in for 90 min. at 25 ºC in dark [42].

The electron donation ability of the methanol extracts has been estimated as follows: the mixture of 0.5 ml 0.2 mM DPPH methanolic solution and 2 ml of methanolic extracts of *Silybum marianum L.* seeds at different concentrations has been left standing for 30 min. at 25 ºC in dark and the absorbance of the resulting solution has been measured at λ = 517 nm. The antiradical activity is expressed as IC_{50} mg/ml, the concentration required to cause a 50 % DPPH inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of seed extract [43].

The decrease of the absorbance at λ = 517 nm of mixture of 13 ml 1 mM DPPH 96 % ethanolic solution and 1 ml *Silybum marianum L.* 96 % ethanolic extract at 25 ºC in dark has been observed [44].

The inhibitory effect of Silymarin has been estimated by measurement of absorbance at λ = 517 nm of mixture of 2 ml 0.002 % DPPH methanol solution with 2 ml 0.5 mg/ml butanol extracts or with 2 ml 0.5 mg/ml standard Ascorbic acid after 30 min. reaction at 25 ºC in dark [45].

The determination of radical scavenging activity against DPPH has been obtained by measurement of the absorbance at λ = 517 nm after 30 min. at 25 ºC in dark reaction between 3 ml 0.004 % DPPH methanol solution and 3 ml of *Silybum marianum L. m*ethanol extract [46].

The radical scavenging ability of the *Silybum marianum L.* extracts has been determined according to the procedure: micture of 2 ml 0.004 % DPPH ethanol solution and 2 ml of *Silybum marianum L*. ethanol extract has been incubated for 30 min. at room temperature 25 ºC in dark and absorbance has been detected at λ = 517 nm, by using of Butulhydroxytoluene as positive control [47].

Other modifications of DPPH method of Blios include the changing of reation time, proportions between volumes of DPPH solution and extracts and absorption maximum.

For the enhancement of Silymarin and phenolic compound accumulation in tissue culture of Milk thistle, the DPPH test with minor modifications has been utilized. In this modificated method by dissolving of 22 mg of DPPH in 50 ml methanol has been obtained 1 mM stock reagent solution and has been stored at −20 °C until use. By dilution of 6 ml of stock solution with 100 ml of methanol has been prepared 0.06 mM working solution. 0.1 ml of extracts of different concentrations and 0.1 ml of synthetic antioxidant Butulhydroxytoluene solution has been vortexed with 3.9 ml 0.06 mM DPPH for 30 s and after 30 min. incubation at 25 ºC in dark, the absorbance of the control, containing all reagents without extract ($A_{control}$) and rhe absorbance of the test compound (A_{sample}) have been recorded at λ = 515 nm. Scavenging activity has been calculated as follows [48]:

DPPH radical-scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \cdot 100$.

In modification of Mekky *et al*. 1 mM DPPH solution (0.394 mg/ml) in methanol has been diluted 1 : 10 to 100 μM solution, which has been reacted with 100 μl of *Silybum marianum L* extract, by using as negative control a mixture of 100 μl methanol and 100 μl DPPH solution. Absorbance has been measured at λ = 515 nm, after incubation for 15 min. at 25 ºC in dark [29].

DPPH radical scavenging activity of methanolic root extracts of *Silybum marianum L.* has been determined by application of the modified method of Blois, in which a mixture of 190 μl 0.15 mM DPPH methanol solution and 10 μl methanol extracts at different concentrations (1 μg/ml – 100 μg/ml) has been incubated for 30 min. at 25 ºC in dark the and the absorbance of the reaction solution at λ = 520 nm has been measured [49].

Free radical-scavenging activity of commercial milk thistle food supplements has been carried out by using microplate reader, performed in a 96 well plate with a total volume of 200 μl methanol, containing 0.004 μg DPPH and samples aliquots at a series of concentrations of 1 μg/ml, 10 μg/ml, 20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml, 200 μg/ml, 400 μg/ml, 800 μg/ml and 2000 μg/ml. DPPH solutions at the same concentration without the tested samples have been used as control. Plates have been incubated in for 30 min. at 25 ºC in dark, followed by reading the absorbance at λ = 520 nm [50].

CONCLUSION

For the estimation of free radical scavenging activity of *Silybum marianum L.* extracts, different modifications of DPPH method has been applied by changing of:

1. Reaction time (from 30 min. to 90 min.)
2. solvent for DPPH (from methanol to ethanol)
3. concentration of DPPH (from 0.1 mM to 0.006 mM, 1 mM, 0.15 mM, 0.2 mM, 0.3 mM)
4. Proportion between volumes of DPPH and extracts.

For different modifications of DPPH method for the assessment of free radical scavenging activity of *Silybum marianum L* .extracts, the following most important conclusions can be summarized:

The most of ten applied modifications are in connection with the changing of

1. concentration of DPPH solution: 0.1 mM to 0.006 mM, 0.15 mM, 0.2 mM, 0.3 mM, 1 mM
2. proportion between volumes of DPPH and extracts

The most of ten used conditions of DPPH method are

1. 30 min. reaction time between DPPH solution and extracts
2. using of methanol as solvent
3. absorption maximum: λ = 517 nm.

References

1. Kroll, D.J., Shaw, H.S. and Oberlies, N.H. 2007. Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies. *Integr. Canc. Ther.*, 6(1): 110-111.
2. Tůmová, L., Řimáková, J., Tůma, J. and Dušek, J. 2006. *Silybum marian*um in vitro-flavonolignan production. *Plant Soil Environment.*, 52(10): 454-458.
3. Mac Kinnon, S.L., Hodder, M., Craft, C. and Simmons-Boyce, J. 2007. Silyamandin, a new flavonolignan isolated from milk thistle tinctures. *Planta Med.*, 73(11): 1214-1216.
4. Alikaridis, F., Papadakis, D., Pantelia, K. and Kephalas, T. 2000. Flavonolignan production from *Silybum marianum* transformed and untransformed root cultures. *Fitoterapia*, 71(4): 379-384.
5. Hackett, E.S., Twedt, D.C. and Gustafson, D.L. 2013. Milk thistle and its derivative compounds: a review of opportunities for treatment of liver disease. *J. Vet. Intern. Med.*, 27(1): 10-16.
6. Viktorova, J., Stranska-Zachariasova, M., Fenclova, M., Vitek, L., Hajslova, J. *et al*. 2019. Complex evaluation of antioxidant capacity of Milk thistle dietary supplements. *Antioxidants*, 8(8): 1-18.
7. Surai, P.E. 2015. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. *Antioxidants*, 4(1): 204-247.
8. Ghosh, A., Ghosh, T. and Jain, S. 2010. Silymarin – a review on the pharmacodynamics and bioavailabilyty enchancemeut approaches. *J. Pharm. Sci. Technol.*, 2(10): 348-355.
9. Trappoliere, M., Caligiuri, A., Schmid, M., Bertolani, C., Failli, P. *et al*. 2009. Silybin, a component of Sylimarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells. *J. Hepatol.*, 50(6): 1102-1111.
10. Qin, N., Sasaki, T., Li, W., Wang, J., Zhang, X. *et al*. 2018. Identification of flavonolignans from *Silybum marianum* seeds as allosteric protein tyrosine phosphatase 1B inhibitors. *J. Enzyme Inhibit. Med. Chem.*, 33(1): 1283-1291.
11. Voroneanu, L., Nistor, I., Dumea, R., Apetrii, M. and Covic, A. 2016. Silymarin in type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *J. Diabet. Res.*, 1: 1-10.
12. Ebrahimpour,K.S., Gargari, B.P., Mobasseri, M., Valiza deh, H. and Asghari-Jafarabadi M. 2015. Effects of *Silybum marianum L. Gaertn.* (Silymarin) extract supplementation on antioxidant status and hs-CRP in patients with type 2 diabetes mellitus: a randomized, triple-blind, placebo-controlled clinical trial. *Phytomed.*, 22(2): 290-296.
13. Singh, U. and Jialal, I. 2006. Oxidative stress and atherosclerosis. *Pathophysiol.*, 13(3): 129-142.
14. Losada-Barreiro, S. and Bravo-Díaz, C. 2016. Free radicals and polyphenols: the redox chemistry of neurodegenerative diseases. *Eur. J. Med. Chem.*, 133(16): 379-402.
15. Guidi, I., Galimberti, D., Lonati, S., Novembrino, C., Bamonti, F. *et al*. 2006. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol. Aging*, 27(2): 262-269.
16. Arteel, G.E. 2003. Oxidants and antioxidants in alcohol induced liver disease *Gastroenterol.*, 124(3): 778-790.
17. Firuzi, O., Spadaro, A., Spadaro, C., Riccieri, V., Petrucci, R., *et al*. 2008. Protein oxidation markers in the serum and synovial fluid of psoriatic arthritis patients. *J. Clin. Lab. Anal.*, 22(3): 210-215.
18. García-Pérez, P, Lozano-Milo, E., Gallego, P.P., Tojo, C., Losada-Barreiro S. and *et al*. 2018. Plant antioxidants in food emulsions. In: Dispersed Food Systems, In Tech Open, 1-30.
19. Cai, Y., Luo, Q., Sun, M. and Corke, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.,* 74(17): 2157-2184.
20. Ahmad, N., Fazal, H., Abbasi, B.H., Anwar, S. and Basir, A. 2012. DPPH free radical scavenging activity and phenotypic difference in hepatoprotective plant (*Silybum marianum L.*) *Toxycol. Industr. Health*, 29(5): 460-467.
21. Elwekeel, A., Zid, S.A, Sokkar, N. and Elfishway, A. 2012. Studies on flavanolignans from cultured cells of *Silybum marianum. Acta Physiolog. Plantarum,* 34(4): 1445-1449.
22. Hogan, F.S., Krishnegowda, N.K., Mikhailova, M. and Kahlenberg, M.S. 2007. Flavonoid, Silibinin, inhibits proliferation and promotes cell-cycle arrest of human colon cancer. *J. Surgic. Res.*, 143(1): 58-65.
23. Alam, M.N., Bristi, N.J. and Rafiquzzaman, M. 2013. Review on in-vivo and in-vitro methods evaluation of antioxidant activity. *Saudi Pharm. **J.***, 21(2): 143-152.
24. Joseph, N.M., Sabhrwal, M., Shahsi, A., Mahor, A. and Rawal, S. 2010. In vitro and in-vivo models for antioxidant activity evaluation: a review. *Int. J. Pharm. Sci. Res.*, 1(1): 1-11.
25. Kaushik, V., Chaudhary, G., Ahmad, S. and Saini, V. 2015. Assays for natural antioxidant. *Global. J. Biol. Agric. Health. Sci.*, 4(3): 130-136.
26. Badarinat, A.V., Rao, K.M., Chetty, C.M.S., Ramkanth, V., Rajan, T.V.S. *et al*. 2010. A review on in-vitro antioxidant methods: comparisons, correlations and considerations. *Int. J. Pharm. Tech. Res.*, 2(2): 1276-1285.
27. Chanda, S. and Dave, R. 2009. In-vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. *Afr. J. Microbiol. Res.*, 3(13): 981-996.
28. Srivalli, R., Kumari, A.B., Maheswari, U.K., Prabhakar, N.B. and Suneetha, J.W. 2016. Analysis of antioxidant activity in pusa ruby tomato podwer incorporated cold

extrudates. *Int. J. Rec. Adv. Multidiscipl. Res.*, 3(10): 1896-1899.

29. Mekky, H., El-Sohafy, S., El-Khair, R.A. and El-Hawiet, A. 2017. Total polyphenolic content and antioxidant activity of *Silybum marianum L.* ciultures grown on differennt growth regulators. *Int. J. Pharm. Pharm. Sci,*, 9(4): 44-47.
30. Blois, M.S. 1958. Antioxidant determination by the use of a stable free radical. *Nat.*, 181: 1199-1200.
31. Rangamanaicker, P., Selvi, T.S.S., Samuvel, T.J., Senguttuvan, J. and Subramaniam, P. 2016. Evaluation of antioxidant and antiinflammatoty properties of the traditional medicinal plant *Cryptolepis grandiflora Wigth* (Apocynaceae). *Int. J. Rec. Adv. Multidiscipl. Res.*, 3(3): 1295-1301.
32. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autioxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.*, 40(6): 945-948.
33. Kumar, N.R., Arumugasamy, K., Sekar, T., Harikrishnen, N., Kaffoor, A.H. *et al*. 2015. In vitro antioxidant activity of *Hydrocotyle conferta* wigth (Apiaceae) – an endangered plant species on southern western ghats. *Int. J. Rec. Adv. Multidiscipl. Res.*, 2(12): 1102-1104.
34. Varsha, V., Suresh, S.N. and Prejeena, V. 2017. Phytochemical screening, antioxidant property and anticancer potential against MCF-7 cell lines of *Vernonia cinerea* leaves. *Int. J. Rec. Adv. Multidiscipl. Res.*, 4(2): 2335-2341.
35. Prejeena, V., Suresh, S.N. and Varsha, V. 2017. Phytochemical screening, antioxidant analysis and antiproliferative effect of *Costus pictus* d. don leaf extraxts. *Int. J. Rec. Adv. Multidiscipl. Res.*, 4(3): 2373-2378.
36. Rajesh, P.M. and Natvar, P.J. 2011. In vitro antioxidant activity of coumarin compounds by DPPH, super oxide and nitric oxide free radical scavenging methods. *J. Adv. Pharm. Edu Res.,* 1(1): 52-68.
37. Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.*, 28(1): 25-30.
38. Ekezie, F.G.C., Suneetha, J.W., Maheswari, U.K., Prasad, T.N.V.K.V. and Kumari, A.B. 2015. Analysis of antioxidant potential of *Momordica charantia* (Bitter gourd) in-vitro. *Int. J. Rec. Adv. Multidiscipl. Res.*, 2(11): 989-992.
39. Köksal, E., Gülçin, İ, Beyza, S., Sarikaya, Ö. and Bursal, E. In-vitro antioxidant activity of Silymarin. *J. Enzyme Inhib. Med. Chem.*, 24(2): 395-405.
40. Serçe, A., Toptancı, B.C., Tanrıkut, S.E., Altaş, S., Kızıl G. *et al*. 2016. Assessment of the antioxidant activity of *Silybum marianum* seed extract and its protective effect against DNA oxidation, protein damage and lipid peroxidation. *Food Technol. Biotechnol*., 54(4): 455-461.
41. Arampatzis, D.A., Karkanis, A.C. and Tsiropoulos, N.G. 2019. Silymarin content and antioxidant activity of seeds of wild *Silybum marianum* populations growing in Greece. *Annals Appl. Biol.*, 174(1): 61-73.
42. Asghar, Z. and Masood, Z. 2008. Evaluation of antioxidant properties of Silymarin and its potential to imhibit peroxyl radical in-vitro. *Pak. J. Pharm. Sci.*, 21(3): 249-254.
43. Mhamdi, B., Abbassi, F., Smaoui, A., Abdelly, C. and Marzouk, B. 2016. Fatty acids, essential oil and phenolics composition of *Silybum marianum* seeds and their antioxidant activities. *Pak. J. Pharm. Sci.*, 29(3): 951-959.
44. Hadaruga, D.I. and Hadaruga, N.G. 2009. Antioxidant activity of hepatoprotective Silymarin and *Silybum marianum L.* extract. *Chem. Bull. Politehnica Univ. (Timisoara)*, 54(68, Suppl. 2): 104-107.
45. **Abdelazim, S.A.A.** 2017. Effect of Silymarin as natural antioxidants and antimicrobial activity**.** *Nutr. Food Sci. Int. J.*, 2(3): 1-6.
46. Ibrahim, G.E., El-Raey, M.A., Eldahshan, O.A. and Souleman, A.M.A. 2013. Effect of extraction on phenolic content, Sylimarin and antiradical activities of Artichoke leaves and roots. *Eur. Sci. J.*, 9(6): 100-110.
47. Sun, J., Li, X.H. and Yu, X.L. 2016. Antioxidant activities, total flavonoids and phenolics content in different parts of *Silybum marianum L.* plants. *Chem. Eng. Transact.*, 55(1): 37-42.
48. Gabr, A.M.M., Ghareeb, H., El-Shabrawi, H.M., Smetanska, I. and .Bekheet, S.A. 2016. Enhancement of silymarin and phenolic compound accumulation in tissue culture of Milk thistle using elicitor feeding and hairy root cultures. *J. Genetic Eng. Biotechnol.*, 14(2): 327-333.
49. Riasat, R., Riasat, Z., Abbasi, B.H., Liu, C. and Khan, M.A. 2015. *Silybum marianum*: adventitious roots induction along with free radical scavenging activity. *J. Plant. Biol. Res.*, 4(1): 12-21.
50. Anthony, K. and Saleh, M.A. 2012. Chemical profiling and antioxidant activity of commercial milk thistle food supplements. *J. Chem. Pharm. Res.*, 4(10): 4440-4450.

How to cite this article:

Danka Obreshkova *et al* (2020) 'Comparison of Different Modifications of Dpph Method for the Estimation of Radical Scavenging Activity of Silybum Marianum (L.)', *International Journal of Current Advanced Research,* 09(01), pp. 21060-21065. DOI: http://dx.doi.org/10.24327/ijcar.2020. 21065.4130

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