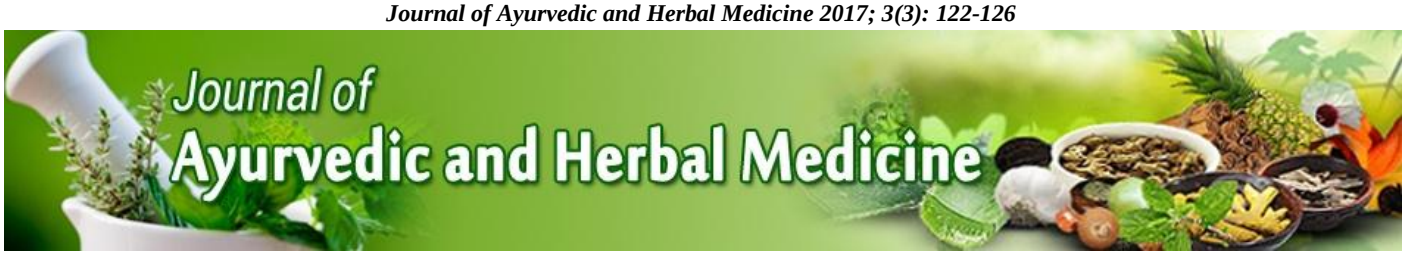

Research Article

ISSN: 2454-5023
J. Ayu. Herb. Med.
2017; 3(3): 122-126

www.ayurvedjournal.com
Received: 29-07-2017
Accepted: 02-09-2017

***Corresponding author:**
Dr. Venkatachalapathi A
PG and Research Department of Botany,Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu-641029, India
Email:
avenkatachalapathi61[at]gmail.com

In vitro antioxidant activity and polyphenol estimation of methanolic fruit extract of *Carissa spinarum* L.

Venkatachalapathi A[1], Abdul Kaffoor H[1], Paulsamy S[1]

1 *PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu- 641029, India*

ABSTRACT

Antioxidant property of methanolic fruit extract of the medicinal tree species, *Carissa spinarum* was evaluated by studying the contents of total phenolics, tannins and flavonoids, free radical scavenging activity using 1,1-diphenyl-2-picryl hydrozyl (DPPH), hydroxyl radical scavenging activity, reducing power activity, $ABTS^{\bullet+}$ assay and metal chelating activity. The results of the study revealed that both the parts studied were found to have potent antioxidant activity against DPPH, hydroxyl and $ABTS^{\bullet+}$ radicals with the IC_{50} value of 88.98 for methanolic fruit extract for DPPH radicals and 849.70 for hydroxyl radicals. Therefore methanolic fruit extract of *C. spinarum* can be considered as a new potential source of natural antioxidants for pharmaceutical industries.

Keywords:*Carissa spinarum*, free radical scavenging activity (DPPH), hydroxyl radical scavenging activity, reducing power activity, $ABTS^{\bullet+}$, metal chelating activity.

INTRODUCTION

Antioxidants, which scavenge active oxygen species (free radicals) are found in a variety of food stuffs and are commonly referred to as scavengers. Many antioxidants are plant based and play an important role in protecting plants against strong sunlight and live under severe oxygen stress and also in human health care measures [1-4]. According to recent research, activated oxygen is thought to be a major factor in ageing, hardening of the arteries, diabetes, cancer and tissue injury of skin. Indeed approximately 90% of age related diseases are linked to activated oxygen.

Recently, there has been a considerable interest in finding natural antioxidant from plant materials to replace synthetic ones. Natural antioxidant substances are presumed to be safe since they occur in plant foods, and are seen as more desirable than their synthetic counter parts [5]. Typical compounds that exhibit antioxidative activity include vitamins, carotenoids, and phenolic compounds [6-11]. Exploration of plant species for antioxidant property is most essential for human health care. From the ethnobotanical survey made in the present study, it is known that the Irula tribal healers of Walayar valley area prescribing the fruits of *Carissa spinarum* (Apocynaceae) for getting freshness. Therefore, in order to know the antioxidant property of this species, fruits were attempted in the present study. It is the indigenous species of southern India distributed in Walayar valley, the Western Ghats of Coimbatore district of Tamil Nadu, and Anantapur, Chittur and Cuddapah districts of Andrpradesh[12]. The leaves and fruits of these plants are used for the treatment of antidiabetic and antiarthritic in the traditional medical practice of tribal in Tamil Nadu and Andra Pradesh [13].

MATERIALS AND METHODS

Plant material

The ripened fruits of *C. spinarum* was collected in moist deciduous forests of Walayar valley, the Western Ghats of Tamil Nadu, India.

Preparation of extracts

The shade dried fruits of the study species were made into fine powder of 40 mesh size using the pulvarizer separately. Hundred grams of the powder was filled in the filter paper and successively extracted by using 500mL methanol in soxhlet extractor for 8 to 10 hours [14]. Then the extract was filtered through Whatman No. 1 filter paper to remove all undissolved matter, including cellular material and other constituents that are insoluble in the extraction solvent.

Determination of total phenolic and tannin contents

The total phenolic content was determined according to the method described by Siddhuraju and Becker [15]. Aliquots of each extract were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents (GAE). Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP). Hundred mg of PVPP was weighed in a 100×12 mm test tube and to this 1.0 ml of distilled water and then 1.0 ml of tannin containing phenolic extract were added. The content was vortexed and kept in the test tube at 4ºC for 4 h. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured, as monitored above and expressed as the content of non-tannin phenolics on dry matter. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) - Non-tannin phenolics (%).

Estimation of total flavonoid content

The total flavonoid content of samples was determined by following the modified colorimetric method of Zhishen*et al.*[16]. 0.5 ml extract was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 ml of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was recorded at 510 nm versus prepared water blank. Rutin was used as a standard compound for the quantification of total flavonoid. All the values were expressed as milligram of rutin equivalent (RE) per gram of extract.

DPPH radical scavenging activity

The 2, 2-diphenyl-picryl-1-picryl-hydrazyl radical (DPPH) scavenging activity was measured according to the method of Blois [17]. Methanolic fruit extract of the samples at various concentrations (100, 150, 200, 250 and 300 µg/mL) was added separately to each 5mL of 0.1mM methanolic solution of DPPH and allowed to stand for 20min. Absorbance at 517nm using spectrophotometer was measured. BHT was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

DPPH radical scavenging activity (%) =
[Control OD-Sample OD/Control OD] ×100

IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical/OH^+ radical which has been determined by using the software SPSS v.16.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of methanolic fruit extract of *Carissa spinarum* was determined by following the method of Zhao *et al.*[18]. Reaction mixture prepared for this experiment contained 500 µL of $FeSO_4$, 500µL of phenanthroline, 2.5mL of PO_4 buffer (pH 7.8), 500µL of H_2O_2 and respective concentrations of fruit samples at 650, 700,750, 800 and 850 µg/mL and the reaction was started by adding H_2O_2. After incubation at room temperature for 5min, the absorbance at 536nm was measured. The hydroxyl radical scavenging activities were calculated similarly to that of DPPH radical scavenging activity.

Assay of Reducing Power

Reducing power assay was determined according to the method of Yildirim*et al.*[19]. Different concentrations of methanolic fruit extract of the study species (100, 150, 200, 250 and 300 µg/mL) were mixed with 2.5mL of 200mM sodium potassium ferric cyanide and incubated at 500C for 20 min. After adding 2.5mL of 10% trichloro acetic acid, the mixture was centrifuged at 3000rpm for 10min. The supernatant was taken out and immediately mixed with 5mL of distilled water and 0.5mL of 1% ferric chloride. After incubation for 10min. the absorbance was measured at 700nm. Higher absorbance of the reaction mixture indicates reductive potential of the extract.

Antioxidant activity by the $ABTS^{\bullet+}$ assay

The total antioxidant activity of the samples was measured by $ABTS^{\bullet+}$ [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical cationdecolorization assay according to the method of Re *et al.*[20]. $ABTS^{\bullet+}$ was produced by reacting 7mM $ABTS^{\bullet+}$ aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance of 0.700 ± 0.02 at 734 nm. The stock solution of the sample extracts was diluted such that after the introduction of 10 µl aliquots into the assay, which have been produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 ml of diluted $ABTS^{\bullet+}$ solution to 10 µl of sample or trolox standards (final concentration 0-15 µM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated from the blank absorbance at 734 nm and then it was plotted as a function of trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of trolox having equivalent antioxidant activity expressed as µmol/g sample extract on dry matter.

Metal chelating activity

The chelating activity of ferrous ions by methanolic fruit extracts of *C. spinarum* was estimated by the method of Dinis*et al.*[21]. Briefly the extract samples (250 µl) were added to a solution of 2 mmol/L $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Statistical analysis

All analyses were carried out in triplicate and the data were reported as mean ± SD. The data were subjected to one way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's Multiple Range Test ($P<0.05$) using the statistical software Inc., Tulsa, OK, USA.

RESULTS

Total phenolic and tannin compounds

The total phenolic content of methanolic fruit extracts of the study species, *C. spinarum* is found to be 2256.26mg GAE/g extract respectively in terms of gallic acid equivalent (Table 1). Similarly, the

tannin content of the study species *C. spinarum* gave higher yield of 885.81mg GAE/g (Table 1).

Table1: Content of total phenolics, tannins and flavonoids content in methanolic fruit extract of *C. spinarum*.

S. No.	Sample	Total phenolics (mg GAE/g extract)	Total tannin (mg GAE/g extract)	Total flavonoids (mg RE/g extract)
1.	Fruit	2256.26±10.74	885.81±9.30	0.13±0.01

GAE-Gallic acid equivalent, RE-Rutin equivalent.

Total flavonoid compound

The total flavonoid content of methanolic fruit extract was determined to be 0.13mg RE/g Rutin Equivalent/g extract (Table 1).

Free radical scavenging activity (DPPH method)

In the present study, the percentage of scavenging effect on the DPPH radical was increased with the increase in the concentration of fruit extract from 100 to 300µg/mL by showing the per cent inhibition from 53.16 at 100µg/mL to 89.15 at 300µg/mL for fruit extract (Table 2). The IC_{50} value of fruit extract was 88.98 over the standard, BHT, 28.12. From the results it is known that the species, *C. spinarum* possess hydrogen donating capabilities and does scavenging free radicals.

Table 2: Free radical scavenging activity (DPPH method) of methanolic fruit extract of *C. spinarum*.

S. No.	Fruit		
	Sampleconcentration(µg/ml)	Percentageactivity	IC_{50}(µg/ml)
1.	100	53.16^{e}± 0.40	88.98
2.	150	74.09^{d}± 0.82	
3.	200	84.82^{bc}±0.49	
4.	250	87.60^{b}± 1.63	
5.	300	89.15^{a}± 0.65	

IC_{50} for the standard BHT = 28.12.
Values are expressed as mean±SD (n=6).
Values within the same column not sharing common superscript letters (a-e) differ significantly at p<0.05 by DMRT.

Hydroxyl radical scavenging activity

Table 3 shows the OH^{+} scavenging effects of fruit extract of *C. spinarum* at different dose levels such as 650, 700, 750, 800 and 850µg/mL. Fruit sample generally registered good hydroxyl radical scavenging activity and it was concentration dependant. Among them, the methanol extract of fruit showed the highest OH^{+} scavenging potential as the IC_{50} value, 849.30µg/mL. The ability of the *C. spinarum* extracts to quench hydroxyl radicals seems to be appreciable and hence the extracts are good scavengers of active oxygen species, thus reducing the rate of chain reaction.

Table 3: Hydroxyl radical scavenging activity of methanolic fruit extract of *C. spinarum.*

S. No.	Fruit		
	Sampleconcentration(µg/ml)	Percentageactivity	IC_{50}(µg/ml)
1.	650	18.20^{e}± 0.82	849.30
2.	700	22.02^{d}± 0.49	
3.	750	39.85^{bc}± 0.65	
4.	800	42.76^{b}± 0.82	
5.	850	48.85^{a}± 0.33	

IC_{50} for the standard BHT = 28.69.
Values are expressed as mean±SD (n=6).
Values within the same column not sharing common superscript letters (a-e) differ significantly at p<0.05 by DMRT.

Reducing power activity

Reducing power activity is based on the principle that substances which has reduction potential, react with potassium ferric cyanide (Fe^{+3}) to form potassium ferrous cyanide (Fe^{+2}), which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm. Methanolic fruit extract of *C. spinarum* in which the reducing power of the extract was increasing with the increase in concentrations. The reducing power of methanolic fruit extract of *C. spinarum* is comparable to that of standard, ascorbic acid (Table 4).

Table 4: Reducing power assay of methanolic fruit extract of *C. spinarum.*

S. No.	Sample/Standard Concentration (µg/ml)	Absorbance at 700nm	
		Methanolic fruit extract	Standard ascorbic acid
1.	100	0.24^{e}±0.09	0.48^{e}±0.08
2.	150	0.38^{d}±0.06	0.59^{d}±0.02
3.	200	0.47^{bc}±0.08	0.63^{c}±0.05
4.	250	0.48^{b}±0.04	0.74^{b}±0.05
5.	300	0.49^{a}±0.08	0.86^{a}±0.03

$ABTS^{\bullet+}$cation radical scavenging activity

The study for the species, *C. spinarum* reports that the methanolic fruit extract of the species registered the highest total antioxidant activity, 2153.79µmol/g (Table 5) which indicates that the study species have considerable radical scavenging activity.

Metal chelating activity

The chelating effect on ferrous ions by the methanolic fruit extract of *C. spinarum* is presented in Table 5. The sample exhibited the ability to chelate metal ions (158.80mg EDTA/g).

Table 5: ABTS and metal chelating activity of methanolic fruit extract of *C. spinarum*.

S. No.	Sample	Total antioxidant activity (µmol TE/g extract)	Metal chelating activity (mg EDTA/ g extracts)
1.	Fruit	2153.79±2.34	158.80±2.01

Total antioxidant activity (µmol equivalent trolox performed by using ABTS·+ radical cation).

DISCUSSION

Total phenolic and tannin compounds

Phenolic compounds are known as powerful chain breaking antioxidants [22] and they are very important plant constituents because of their scavenging ability by their hydroxyl groups [23]. These compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity [24]. The phenolic compounds may contribute directly to antioxidative action. It was suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested upto 1gm daily from a diet rich in fruits and vegetables [25]. The tannin containing remedies are used as antioxidants [26] in addition to number of other therapeutic uses.

Total flavonoid compound

Flavonoids present in food of plant origin are also potential antioxidants [27,28]. Most of the beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities [29]. Studies have shown that certain flavonoids exhibit hypoglycaemic effect also [30].

Free radical scavenging activity (DPPH method)

DPPH is a stable free radical that accepts an electron hydrogen radical to become a stable diamagnetic molecule [31]. The decrease in absorbance of DPPH radical caused by antioxidants, because of reaction between antioxidant molecules and radicals, results in the scavenging of radical by hydrogen donation [32]. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidants [33,34].

Hydroxyl radical scavenging activity

Scavenging of OH^+ is an important antioxidant activity because of its very high reactivity which can easily cross the cell membranes at specific sites, react with the biomolecules and further causes cell damage and finally cell death. Thus, removing of OH^+ is most important for the protection of living system [35].

Reducing power activity

The reducing capacity of the study species may serve as a significant indicator of its potential antioxidant activity as reported for the species, *Rumexcrispus* by Yildirim*et al.*[19].

$ABTS^{\bullet+}$cation radical scavenging activity

$ABTS^{\bullet+}$, a protonated radical has characteristic absorbance maximum at 734nm which decreases with the scavenging of the proton radicals. $ABTS^{\bullet+}$ was generated by incubating $ABTS^{\bullet+}$ [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] with potassium persulfate. The presence of chemical compounds in the tested extracts that inhibit the potassium persulfate activity may reduce the production of $ABTS^{\bullet+}$.

Metal chelating activity

Iron is essential for life system because it is required for oxygen transport, respiration and activity of many enzymes. In complex systems such as food and food preparation many different mechanisms may contribute to oxidative process such as Fenton reaction, where transition metal ions play a vital role. Different reactive oxygen species might be generated and due to which various target structures like lipids, proteins, carbohydrates etc. can be affected. Therefore, it is important to characterize the extracts by a variety of antioxidant assays [36].Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation [37]. It was already reported that chelating agents which form ó bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, there by stabilizing the oxidized form of the metal ion [38]. Antioxidants inhibit interaction between metal and lipid through the formation of insoluble metal complexes with ferrous ion. Hence, the data obtained for the study species revealed that the extract of fruit demonstrate an effective capacity for iron binding and hence the antioxidant property.

CONCLUSION

This research provides information about the antioxidant property of the fruit part of *C. spinarum*. Hence, it is identified that this specie can be used as a source for the manufacturing of drugs of scavenging property. However, large scale *in vivo* studies are required to confirm the scavenging property before going for commercialization.

Source of support – None.

Conflict of interest – Authors have no conflict of Interest.

REFERENCES

1. Badami S, Moorkoth S, Rammanoharsingh RS, Kannan E, Bhojraj S. Antioxidant activity of *Coesalpiniasappan* heart wood. Biol. Pharm. Bulletin. 2003; 26(11):1534-1537.
2. Atawodi SE. Antioxidant potential of African medicinal plants. African J. Biotechnol. 2005; 4(2):128-133.
3. Shyur, Lie-Fen, TsungJieh-hen, Chen Ie-Hsin, Chiu Chih- Yang, Lo Chiu-Ping. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. Int. J. Appl. Sci. Eng. 2005; 3(3):195-202.
4. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2006; 94:550-557.
5. Pratt DE. Natural antioxidant from plant material. *In*: IMT, Huang, CT. HO & CY Lee (eds.). Phenolic compounds in food and their effects on health. New York American Chemical Society, 1992.
6. Morris MC, Bekett LA, Scherr PA, Hebert LE, Bennett DA, Field TS, *et al*. Vitamin E and Vitamin C supplement use and risk of incident Alzheimer disease. *Alzheimer* Dise. Asso. Disor. 1998; 12(3):121-126.
7. Klipstein-Grobusch K, Launer LJ, Geleijnse JM, Boeing H, Hofman A, Witterman JC. Serum carotenoids and antherosclerosis. The Rotterdam Study. *Atherosclerosis*, 2000; 148(1):49-56.
8. Moeller SM, Jacques PF, Blumberg JB. The potential role of dietary xanthophylls in cataract and agerelated macular degeneration. J. American Coll. Nut. 2000; 19(5):5225-5278.
9. Slattery ML, Benson J, Curtin K, Ma KN, Schacffer D, Pootter JD. Carotenoids and colon cancer. American J. Clin. Nut. 2000; 7(2):575-582.
10. Basniwal PK, Suthar M, Rathore GS, Gupta R, Kumar V, Pareek A, Jain D. *In vitro* antioxidant activity of hot aqueous extract of *Helicteresisora* Linn. fruits. Nat. Prod. Rad. 2009; 8(5):483-487.
11. BorokiniTI, Omotayo FO. Phytochemical and ethanobotanical study of some selected medicinal plants from Nigeria. J. Med. Plants Res. 2012; 6(7):1106-1118.
12. Pullaiah T. Medicinal plants in India. Regency Publications, New Delhi, 2000.
13. Fatima A, Singh PP, Agarwal P, Irchhaiya R, Alok S, Verma A. Treatment of various diseases by *Carissa spinarum* L. Int. J. Pharm. Sci. Res. 2013; 4(7):2489-2495.
14. Gafner F, Msonthi JD, Hostettmann K. Mlluscicidalsaponins from *Talinumtenuissimum*Dinter. Helvet. AnalyticaChimica. Acta. 1985; 68:555-558.
15. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of Drumstick tree (*Moringaoleifera* L.) leaves. J. Agricul. Food Chem. 2003; 51(8):2144-2155.
16. Zhishen J, Mengcheng T, Jianming W. Research on antioxidant activity of flavonoids from natural materials. Food Chem. 1999; 64:555-559.
17. Blois MS. Antioxidants determination by the use of a stable free radical. Nature. 1958; 26:1199-1200.
18. Zhao GR, Xiang ZJ, Ye TX, Yuan YJ, Guo ZX. Antioxidant activities of *Salviamiltiorriza* and *Panaxnotoginsing*. Food Chem. 2006; 99:767-774.
19. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumexcrispus* L. extracts. J. Agricul. Food Chem. 2001; 9:4083-4089.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice Evans C. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Rad. Biol. Med. 1999; 26:1231-1237.
21. Dinis TCP, Maderia VMC, Almeida LM. Action of phenolic derivatives (acetoaminophen, salycillate and 5 aminosalycillate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophy. 1994; 315:161-169.
22. Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Food Sci. Nutr. 1992; 32:67-103.
23. Hatano T, Edamatsu R, Mori A. Effect of interaction of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem. Pharm. Bull. 1989; 37:2016-2021.
24. Yen GC, Duh PD, Tsal CL. Relationship between antioxidant activity and maturity of peanut hulls. J. Agricul. Food Chem. 1993; 41:67-70.

25. Tanaka M, Kuei CW, Nagashina Y. Application of antioxidativemaillrad reaction produces from histidine and glucose to sardine products. Nippon Suisan Gakkaishi. 1998; 47:1409-1414.
26. Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, *et al*. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini-Rev. Med. Chem. 2008; 8:436-447.
27. Salah N, Miller NJ, Paganga G, Tijburg L, Bolvoell GP, Rice Evans C. Polyphenolicflavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch. Biochem. Biophy. 1995; 322(2):339-346.
28. Van Acker SABE, Van DVWJF, Bast F. Structural aspects of antioxidant activity of flavonoids. Free Rad. Bio. Med. 1996; 20(3):331-342.
29. Hassig A, Liang WX, Shwabl K, Stampfl K. Flavonoids and tannins plant based antioxidants with vitamin character. Medical Hypo. 1999; 52:471-481.
30. Ahmed M, Akthar MS, Malik T, Gilani AH. Hypoglycaemic action of the flavonoids fraction of *Cuminumnigrum* seeds. Phytother. Res. 2000; 14:103-106.
31. Dreosti IE. Antioxidant phenols in tea, cocoa and wine. *Nutr.* 2000; 16:692-694.
32. Ogawa K, Suzuki K, Okutsu M, Yamazaki K, Shinkai S. The association of elevated reactive oxygen species leves from neutrophils with lowgrade inflammation in the elderly. Immunity and Ageing. 2008; 5(13):1-8.
33. Shah R, Guclu K, Demirata K, Celik SE, IsilBerker K. Comparative evaluation of various total antioxidant capacity assays. Applied to phenolic compounds with te CUPRAC Assay. Molecules. 2007; 12:1496-1547.
34. Chew YL, Goh JK, Lim YY. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from leguminosae family in Peninsular Malasia. Food Chem. 2009; 116:13-18.
35. Yang S, Guo J, Yuan J. *In vitro* antioxidant properties of rutinLebensm. Wiss. Technol. 2008; 41:1060-1066.
36. Halliwell B. Antioxidants the basicswhat they are and how to evaluate them. Advan. Pharmacol. 1997; 38:3-20.
37. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of HarngJyur*Chrysanthemum morifolicum*Romat. LWT - Food Sci. Technol. 1999; 32:269-277.
38. Keowmaneechai E, Mc Clements DJ. Influence of EDTA and citrate on thermal stability of whey protein stabilized oil-in-water emulsions containing calcium chloride. Food Res.Int. 2006; 39:230-239.

HOW TO CITE THIS ARTICLE
Venkatachalapathi A, Kaffoor HA, Paulsamy S. *In vitro* antioxidant activity and polyphenol estimation of methanolic fruit extract of *Carissa spinarum* L. J Ayu Herb Med 2017;3(3):122-126.