The Journal of Phytopharmacology 2018; 7(3): 263-269
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2018; 7(3): 263-269
May- June
Received: 25-03-2018
Accepted: 21-05-2018

Saraswathi K
Karpaga Vinayaga College of Engineering and Technology, Madhuranthagam, Kancheepuram Tamil Nadu– 603 308, India

Rajesh V
Armats Biotek Training and Research Institute, 14/18C, Link road, Mettu street, Maduvinkarai, Guindy, Chennai Tamil Nadu - 600 032, India

Saranya R
Meenakshi College for Women, Kodambakkam, Chennai Tamil Nadu – 600 024, India

Arumugam P
Armats Biotek Training and Research Institute, 14/18C, Link road, Mettu street, Maduvinkarai, Guindy, Chennai Tamil Nadu - 600 032, India

Correspondence:
Arumugam P
Armats Biotek Training and Research Institute, 14/18C, Link road, Mettu street, Maduvinkarai, Guindy, Chennai Tamil Nadu - 600 032, India
Email: saraskri1591[at]gmail.com

GC-MS, phytochemical analysis and *in vitro* antioxidant activities of leaves of *Canavalia cathartica* Thouars

Saraswathi K, Rajesh V, Saranya R, Arumugam P*

ABSTRACT

Plants have been used for thousands of years to flavour and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of the anti-oxidant properties has been a promising method of assessing free radicals damage. The aim of the present study was to evaluate the antioxidant activities of leaves of *Canavalia cathartica* and to identify the bioactive compounds by performing GC-MS analysis resulting in the presence of volatile and semi volatile compounds. The IC_{50} of $DPPH^{\cdot}$ radical, $ABTS^{\cdot+}$ radical cation, Nitric oxide radical scavenging assays were 84.03, 51.18 and 351.78µg/mL concentration respectively. Also, the IC_{50} of Phosphomolybdenum reduction and ferric reducing power assay were 81.53 and 87.64µg/mL concentration respectively. The results of this study portray the effective antioxidant activity of *Canavalia cathartica* and further studies are required to isolate the active compounds from various parts of this species and their mode of action. From the study it can be concluded that the plant might be promising as a curative for many diseases associated with free radicals.

Keywords: Free radicals, Antioxidant, $DPPH^{\cdot}$ assay, $ABTS^{\cdot+}$ radical cation, IC_{50}, GCMS.

INTRODUCTION

Medicinal plants are considered as potential sources of natural bioactive compounds such as antioxidant molecules and secondary metabolites. Naturally available bioactive compounds absorb sunlight and they produce oxygen in high level and secondary metabolite by photosynthesis. Phenolic compounds and flavonoids are the most important secondary metabolites in plants [1]. *Canavalia cathartica*, commonly known as maunaloa is a species of flowering plant in the legume family, Fabaceae. It has a paleotropical distribution, occurring throughout tropical regions in Asia, Africa, Australia and many Pacific Islands. *Canavalia cathartica* is a biennial or perennial herb with thick, twining, climbing stems. The pinnate leaves are each divided into three papery leaflets which are generally oval in shape with pointed or rounded tips. The inflorescence is a raceme or pseudo-raceme of several flowers [2, 3].

The seeds and pods are used as famine foods in coastal India. It's considered to be an underutilized wild plant with the potential to serve as a protein and carbohydrate rich food crop. When compared to edible legumes, it has rich protein content. It grows rapidly, tolerates challenging habitat types such as sandy, saline soils, etc. *Canavalia cathartica* contains anti-nutrients and requires some processing before using it as a food. Farmers use *Canavalia cathartica* as green manure and mulch and host it in their fields for fixation of nitrogen [4, 5]. The antioxidant potential and also the effective compounds present in the selected medicinal plant have been studied.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts

The leaves of *Canavalia cathartica* were carefully washed with tap water followed by rinsing in distilled water and air-dried at room temperature for few hours. Then leaves were separated and taken to separate clean place and dried at room temperature for one week. Then they were ground into fine powder and sieved through fine mesh, finally stored in cool and dry place in a clean air-tight container. Extraction of leaf powder with Hexane, Ethyl acetate and methanol was performed by direct method [6].

Scientific Classification of *Canavalia cathartica*

Kingdom	**:**	Plantae
Family	**:**	Fabaceae
Class	**:**	Phaseoleae
Subclass	**:**	Faboideae
Order	**:**	Fabales
Genus	**:**	Canavalia
Species	**:**	*Cathartica*

In vitro* Antioxidant Activity of crude extracts of *Canavalia cathartica

(a) Free radical Scavenging Activity

The antioxidant activity was determined by DPPH scavenging assay [7]. Various concentration of the three different crude extracts from the stock solution were pipetted out in clean test tubes. Freshly prepared DPPH (1,1-Diphenyl-2-picryl hydrazyl) solution (1mL) was added to each tube and the samples were incubated in dark at 37°C for 30 min and read at 517nm. The data were expressed as the percent decrease in the absorbance compared to the control. Ascorbic acid was used as reference compound. The percentage inhibition of radical scavenging activity was calculated as:

%Radical scavenging activity =
[(Control OD-Sample OD)/Control OD] X100.

(b) Phosphomolybdenum assay

Total antioxidant capacity can be calculated by the method described by [8]. Various concentration from the prepared stock solution (1mg/ml) were pipetted out and 1mL of the reagent solution containing 4mM Ammonium molybdate, 28mM Sodium phosphate and 0.6M Sulphuric acid was added, followed by incubation in boiling water bath at 95°C for 90mins. After cooling the sample to room temperature, the absorbance of the solution was measured at 695nm in UV spectrophotometer. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions. Ascorbic acid served as standard. The percentage of reduction was calculated as:

% Phosphomolybdenum reducing potential =
[(Sample OD-Control OD)/Sample OD] X100.

(c) Ferric (Fe^{3+}) reducing power assay

The crude extract was taken in various concentration from the stock solution and was mixed with 2.5mL of phosphate buffer (0.2 M, pH-6.6) and 2.5mL of potassium ferricyanide (1% w/v), and incubated at 50°C for 30minutes. Then, 2.5ml of trichloroacetic acid (10% w/v) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5mL of upper layer solution was mixed with 2.5mL of distilled water and 0.5mL $FeCl_3$ (0.01% w/v) and the absorbance was measured at 700nm [9, 10]. Ascorbic acid served as standard. The percentage of reduction was calculated as:

% Ferric reducing potential =
[(Sample OD-Control OD)/Sample OD] X100.

(d) Nitric Oxide radical scavenging activity

The crude extract from the stock solution was taken in various concentration and 2 mL of 10 mM sodium nitroprusside in 0.5mL phosphate buffer saline (pH-7.4) was mixed with the extract. The mixture was incubated at 25°C for 150 minutes. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid v/v) and incubated at room temperature for 5 minutes. Finally, 1.0 mL naphthyl ethylene diaminedihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 minutes before measuring the absorbance at 546nm was measured with a spectrophotometer. Ascorbic acid served as standard [11]. The nitric oxide radical scavenging activity was calculated as:

%Nitric oxide scavenging potential =
[(Control OD-Sample OD)/Control OD] X100.

(e) $ABTS^{\bullet+}$ (2,2-azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) radical cation scavenging assay

The crude extract from the stock solution was taken in various concentration and this assay was performed according to the method of [12]. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. Fresh ABTS solution was prepared for each assay. Plant extract of varying concentration were allowed to react with 500µL of the ABTS solution for 15 minutes in dark condition. Then the absorbance was taken at 734nm using the spectrophotometer. The $ABTS^{\bullet+}$ radical cation scavenging activity was calculated as:

% of $ABTS^{\bullet+}$ radical cation inhibition =
[(Control OD-Sample OD)/Control OD] X100.

Qualitative phytochemical analysis of *Canavalia cathartica*

Screening of phytochemicals for *Canavalia cathartica* was carried out comparatively using standardized methods [13].

Quantitative estimations of total phenol and flavonoids

Determination of total Phenols

The total phenolic content of *Canavalia cathartica* determined with the Folin-Ciocalteau reagent (FCR). 0.1mL of plant extract was added to 46mL of distilled water followed by 1mL of Folin –Ciocalteau reagent and mixed thoroughly and kept at room temperature for 3min [14]. To that 3mL of 2% (w/v) sodium carbonate was added and periodically shaken for 2 hours and the absorbance was measured at 760nm.

Determination of total flavonoids

0.5 mL of plant extract was weighed and to the extract 1.5 mL of ethanol (95% v/v), 0.1 mL of aluminium chloride (10% w/v), and 0.1mL of potassium acetate (1M), 2.8 mL of distilled water was added [15]. The incubation period was carried out for 30 min and the OD was measured at 415nm.The amount of total flavonoids was expresses as µg QE/ml of sample.

Thin layer chromatography analysis

Thin layer chromatography (TLC) analysis was carried out for methanol extract of *Canavalia cathartica* on silica gel aluminium sheet (Merck Silica gel 60 F254) [16]. The methanol extract were spotted at 0.5 mm above from the bottom of the TLC plate. The spotted TLC plate was placed in a 100mL beaker containing solvent mixture. The chromatogram was developed and the spots were visualized under UV light at 254 nm as well as in iodine vapour. The ratio in which distinct coloured bands appeared was optimized and R_f values were calculated.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Identification of bioactive compounds by Gas chromatography-Mass spectrometry analysis

The presence of active compounds were been confirmed by thin layer chromatography and the compounds were identified using gas chromatography and mass spectrometry (GC-MS) method, (TSQ QUANTUM XLS). The name of the instrument is Gas Chromatography-Mass Spectrometry and the instrument made is of Thermo scientific. The software required for analytical studies is XCALIBUR (ver-2.2). The column size is of TG-5MS (30mX0.25mmX0.25um). The injector temperature and interface temperature (°C) was at 280°C

RESULTS AND DISCUSSION

Collection and preparation of plant sample

The Plant *Canavalia cathartica* was collected from the regions of Thiruvanamalai. The leaves were then separated and shade dried for ten days in a well-ventilated room at 37°c and ground to coarse powder using the mechanical grinder. After 72 hours of extraction, the supernatant was filtered by filter paper and condensed in a rotary evaporator at 50°C, which yields gummy extract. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/10ml as final volume for further analysis.

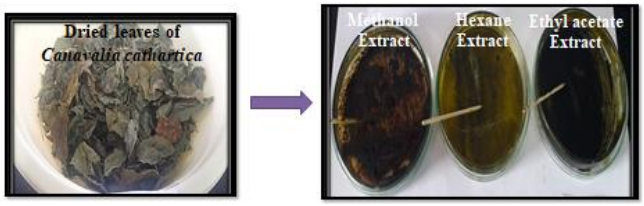

Figure 1: Collection and Preparation of crude extracts of *Canavalia cathartica*

In vitro* Antioxidant Activity of crude extracts of *Canavalia cathartica

(a) Free radical Scavenging Activity

The antioxidant activity was carried out by DPPH assay according to the method of (Blois, 1958). Antioxidant molecules can quench DPPH free radicals (i.e by providing hydrogen atoms or by electron donation, via a free radical attack on the DPPH molecule) and convert them to colorless. The percentage of DPPH scavenging activity was 65.23% in methanol fraction of *Canavalia cathartica* when compared to other two fractions (Table 1). The IC_{50} value was found to be 84.03µg/mL concentration (Figure 2) and was compared with standard (Ascorbic acid, IC_{50} value as 11.98µg/mL concentration).

Table 1: Radical scavenging activity by DPPH assay for different extracts of *Canavalia cathartica*

S. No	Concentration (µg/mL)	Radical Scavenging Activity (%RSA)		
		Hexane extract	Ethyl acetate extract	Methanol extract
1	20	15.33	19.37	18.57
2	40	18.26	28.75	30.02
3	60	23.54	31.6	36.38
4	80	29.18	36.66	47.60
5	100	32.37	39.04	54.19
6	120	38.61	41.2	65.23

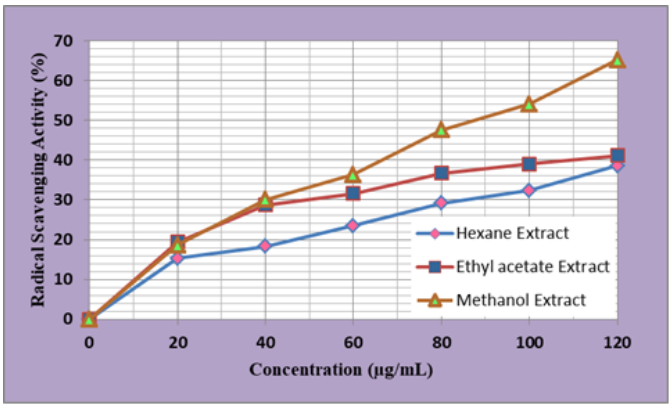

Figure 2: Radical scavenging activity by DPPH assay for different extracts of *Canavalia cathartica*

Based on the screening for the three extracts and also based on the percentage of inhibition by DPPH assay, the optimized solvent (methanol crude extract) was selected for further studies.

(b) Phosphomolybdenum assay

The total antioxidant activity of methanol extract of *Canavalia cathartica* was measured spectrophotometrically by phophomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum reducing ability for methanol extract of *Canavalia cathartica* was 72.85% at 120µg/mL concentration (Figure 3). The experiment demonstrated higher antioxidant activity the IC_{50} of 81.53µg/mL concentration (Table 2) and was compared with standard Ascorbic acid (IC_{50} value as 23.28µg/mL concentration).

Table 2: Phosphomolybdenum Reducing Potential of methanol extract of *Canavalia cathartica*

S. No	Concentration (µg/mL)	Phosphomolybdenum Reducing Potential (%)
1	20	21.39
2	40	38.74
3	60	44.62
4	80	49.06
5	100	68.63
6	120	72.85

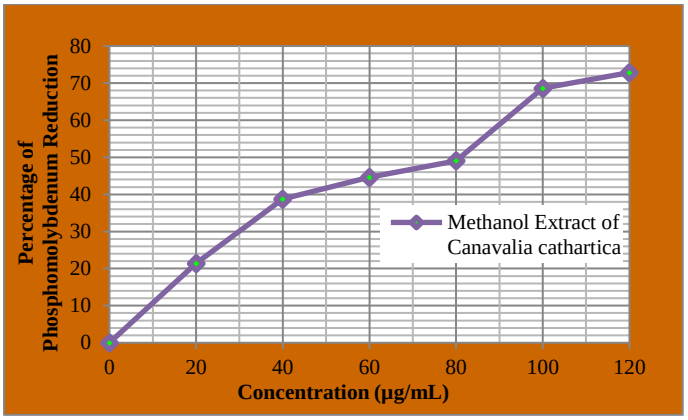

Figure 3: Evaluation of Total antioxidant activity of methanol extract of *Canavalia cathartica*

(c) Ferric (Fe^{3+}) reducing power assay

The antioxidant activity of *Canavalia cathartica* extract was calculated according to the Makari *et al*., 2008, Hennebelle *et al*., 2008. The inhibition in reducing power assay denotes the yellow color of the test solution changes to various shades of green and blue depends upon reducing power of each compound. The maximum reducing ability for methanol extract of *Canavalia cathartica* was 65.16% at 120µg/mL concentration (Figure 4). The IC_{50} value for methanol extract of *Canavalia cathartica* was found to be 87.64µg/mL concentration (Table 3) and was compared with the standard (29.11µg/mL concentration) Ascorbic acid.

Table 3: Fe^{3+} Reducing Potential (FRP) of methanol extract of *Canavalia cathartica*

S. No	Concentration (µg/mL)	Ferric Reducing Potential (%)
1	20	19.07
2	40	31.56
3	60	37.83
4	80	45.64
5	100	61.92
6	120	65.16

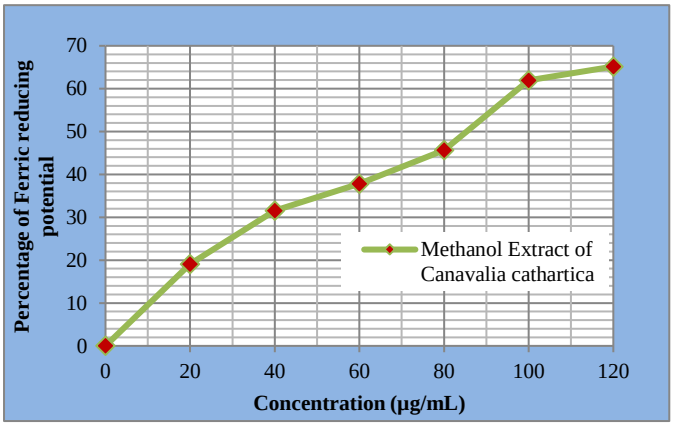

Figure 4: Fe^{3+} Reducing Potential (FRAP) of methanol extract of *Canavalia cathartica*

(d) Nitric Oxide radical scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions. Nitric oxide scavengers compete with oxygen, leading to reduced production of nitrite ions. Large amount of nitric oxide may lead to tissue damage. Nitric oxide is a free diffusible radical. The maximum radical scavenging potential for methanol extract of *Canavalia cathartica* was 42.64% at 300µg/mL concentration (Figure 5). The IC_{50} value for methanol extract of *Canavalia cathartica* was found to be 351.78µg/mL concentration having moderate activity (Table 4) and was compared with the standard (31.14µg/mL concentration) Ascorbic acid.

Table 4: Nitric oxide radical scavenging activity of methanol extract of *Canavalia cathartica*

S. No	Concentration (µg/mL)	Nitric oxide radical scavenging activity (%)
1	50	12.32
2	100	16.69
3	150	18.33
4	200	29.51
5	250	36.78
6	300	42.64

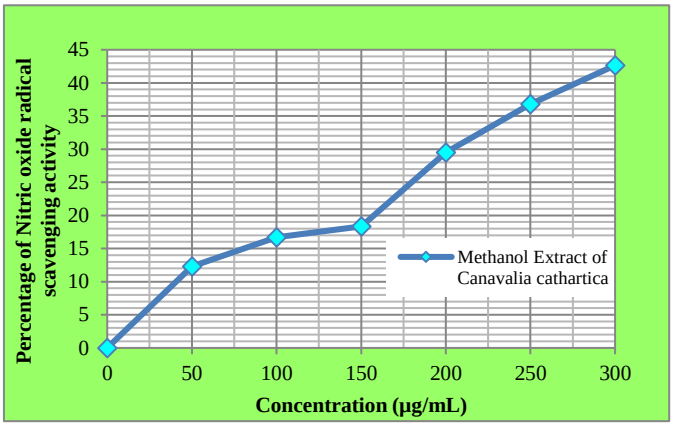

Figure 5: Nitric oxide radical scavenging activity of methanol extract of *Canavalia cathartica*

(e) $ABTS^{\bullet+}$ (2,2-azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) assay

$ABTS^{\bullet+}$ (2,2 – azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) assay measures the relative ability of antioxidant to scavenge the ABTS generated in aqueous phase (Table 5). ABTS is generated by reacting with a strong oxidizing agent (Potassium per sulfate) with ABTS salt. Reduction of blue green ABTS-radical coloured reaction by hydrogen-donating antioxidant is measured at 734nm (Figure 6). The maximum $ABTS^{\bullet+}$ radical cation scavenging activity of methanol extract of *Canavalia cathartica* was found to be 51.59% at 60µg/mL concentration. The IC_{50} value for methanol extract of *Canavalia cathartica* was found to be 51.18µg/mL concentration and was compared with standard Ascorbic acid (IC_{50} value as 4.21µg/mL concentration).

Table 5: ABTS$^{\bullet+}$ assay of methanol extract of *Canavalia cathartica*

S. No	Concentration (µg/mL)	ABTS$^{\bullet+}$ radical cation scavenging activity (%)
1	10	21.43
2	20	27.85
3	30	32.27
4	40	36.52
5	50	48.84
6	60	51.59

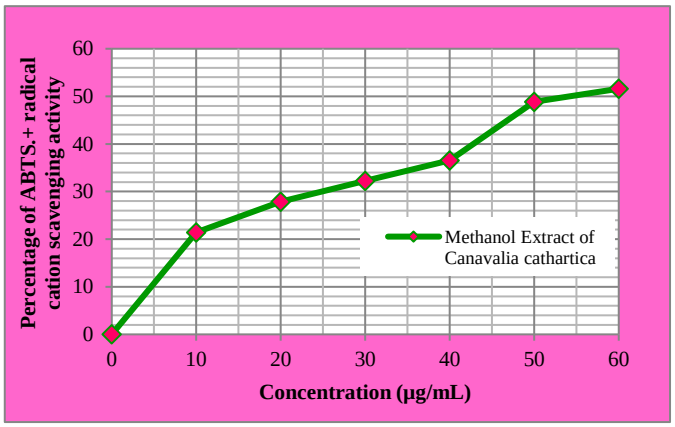

Figure 6: ABTS$^{\bullet+}$ assay of methanol extract of *Canavalia cathartica*

The ABTS$^{\bullet+}$ radical is stable and solubility of ABTS$^{\bullet+}$ is with organic solvents and water, which determines the antioxidant capability of hydrophilic and lipophilic compounds.

Qualitative phytochemical analysis of *Canavalia cathartica*

The results of phytochemical analysis for methanol extract of *Canavalia cathartica* showed the presence of phenols, terpenoids, tannins, steroids and was quantified.

Table 6: Qualitative analysis of methanol extract of C*anavalia cathartica*

S. No	Phytochemicals	Tests	Results
1	Alkaloids	(a) Mayer's test	-
		(b) Hager's test	-
2	Phenols	Ferric chloride (5%) test	+
3	Tannins	Ferric chloride (0.1%) test	+
4	Flavonoids	Sodium hydroxide test	+
5	Glycosides	Legal's test	+
6	Steroids	Libermann-Burchard test	+
7	Terpenoids	Salkowski test	+
8	Saponins	Foam test	-
9	Reducing sugars	Fehling's test	+
10	Proteins	Biuret test	-

Quantitative estimations of total phenol and flavonoids

Total phenol content for methanol extract of C*anavalia cathartica* was 84.93µg/mg of GAE and flavonoid content was 18.50µg/mg of QE.

Table 7: Quantitative estimation of methanol extract of C*anavalia cathartica*

S. No	Phytochemicals	Total amount in plant extract
1.	Flavonoids	18.50 µg/mg
2.	Phenols	84.93 µg/mg

High phenolic rich plant-derived products have the ability to reduce the risk of cardio- cerebrovascular diseases as well as cancer cell death [17]. Tannins, important phyto-constituent have significant effect in scavenging free radicals and reducing oxidative stress [18]. The seeds, pods and leaves of *C. cathartica* are potential source of L-canavanine and have insecticidal property [19, 20, 21]. Young leaves and senescent brownish-green leaves consist of L-canavanine up to 1.63% and 1.3% respectively [22, 23, 24].

Thin Layer Chromatography analysis

Methanol extract of C*anavalia cathartica* was subjected to TLC in order to identify the bioactive compounds. The most appropriate TLC system for analysis was shown to be (Toluene:Ethyl acetate v/v) in the ratio 1.5: 0.5, in which the separation of compounds was most distinct and clear. Five bands (Figure 7) were found with R_f values of 0.81, 0.58, 0.53, 0.18 and 0.11 under Ultra Violet light.

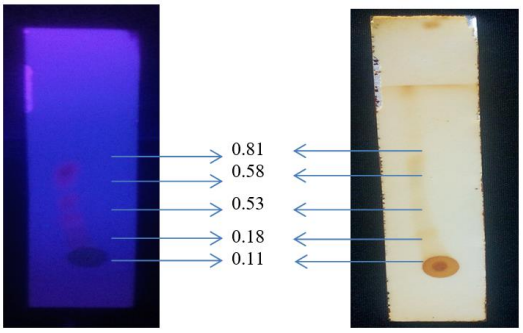

Figure 7: Visualization of bands of methanolic extract of C*anavalia cathartica*

Identification of bioactive compounds by Gas chromatography-Mass spectrometry analysis

GC-MS analysis was carried out for the methanol extract of C*anavalia cathartica* and the eluted compounds were showed in Table 8. Bioactive compounds such as 1,3-Dioxolane, 2,4,5-trimethyl, Catecholborane, Isophytol.

Table 8: GCMS analysis of methanol extract of C*anavalia cathartica*

S. No	Compound Name	Retention Time	Peak Area%	CAS#
1	1,3-Dioxolane, 2,4,5-trimethyl-	12.04	0.33	003299-32-9
2	Catecholborane	12.78	1.79	000274-07-7
3	2-Cyclohexen-1-one, 3 methyl- 6-(1-methylethylidene)-	13.78	0.38	000491-09-8
4	Diethyl Phthalate	16.19	1.60	000084-66-2
5	Thiophene, 2-ethyltetrahydro-2-[2-(2-Ethoxyethoxy)ethoxy] Ethyl acetate	17.24	72.95	001551-32-2
6	2-[2-[2(2-Butoxyethoxy)ethoxy]ethoxy]ethyl acetate	17.29	21.69	1000351-93-9
7	Isophytol	21.41	0.89	000505-32-8

Antioxidants are compounds that are capable to inhibit or slow down the oxidation, which occurs due to environmental stress or Reactive Oxygen Species [25, 26]. Antioxidants are the first line of defense mechanism in neutralizing the free radicals. Fruits, vegetables, grains, nuts, pulses when consumed have a great effect in reducing many chronic diseases [27, 28]. The phytochemicals, derived from medicinal plants have significant role as antioxidant-rich molecules. Excess production of free radicals starts to form chain reactions, circulates in the human system thereby oxidize the low density lipoproteins (LDL) and slowly moving to lethal condition [29, 30]. The oxidation process gets initiated continuously due to specific substances, until a scavenging or neutralizing mechanism occurs [31, 32].

CONCLUSION

From the present investigation, it is evident that the leaves of *Canavalia cathartica* are effective against free radicals which neutralize the environment. The scavenging potential was well observed for DPPH, $ABTS^{\bullet+}$, Nitric oxide activity, Phosphomolybdenum and ferric reducing power assay. Also, the chromatogram developed, suggests that five major compounds are present in the leaf extract of *Canavalia cathartica* which could contribute to its antibacterial activity. These results reveal that the leaves of *Canavalia cathartica* could be a potential source of traditional medicine for infections caused by bacteria and fungi. Further investigation is necessary to elucidate the exact bioactive compound which is responsible for the anti-proliferative action.

Acknowledgement

The authors wish to thank Armats Biotek Training and Research Institute for providing necessary facilities needed for the research.

REFERENCES

1. Kim D, Jeond S, Lee C. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food chem. 2003; 81:321-326.
2. Quattrocchi, Umberto. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. CRC Press, 2012 1:773.
3. Anita DD, Sridhar KR. Assemblage and diversity of fungi associated with mangrove wild legume *Canavalia cathartica*. Tropical and Subtropical Agro-ecosystems. 2009; 10(2):225-35.
4. Seena S, Sridhar KR. Nutritional and microbiological features of little known legumes, *Canavalia cathartica* Thouars and *C. maritima* Thouars of the southwest coast of India, 2006.
5. Bhagya B. Biochemical and protein quality evaluation of tender pods of wild legume *Canavalia cathartica* of coastal sand dunes. Livestock Research for Rural Development. 2006; 18:1-20.
6. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants?, J Ethnopharmacol. 1998; 60(1):1-8.
7. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 29:1199-1200.
8. Prieto P, Pineda M, Anguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal. Biochem. 1999; 269:337-341.
9. Makari HK, Haraprasad N, Patil HS, Ravikumar. *In Vitro* Antioxidant Activity of The Hexane And Methanolic Extracts of *Cordia wallichii* And *Celastrus paniculata*. The Internet J. Aesthetic and Antiaging Medicine. 2008; 1:1-10.
10. Hennebelle T, Sahpaz S, Gressier B, Joseph H, Bailleul F. Antioxidant and Neurosedative Properties of Polyphenols and Iridoids from *Lippiaalba*. Phytotherapy Research. 2008; 22:256-258.
11. Garrat D. The quantitative analysis of drugs, Champman and Hall, Japan, 1964; 3:456-458.
12. Arnao M, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 2001; 73:239-44.
13. Harborne JB. Phytochemical Methods, A guide to Modern Techniques of Plant analysis, second ed. Chapman and Hall, London. 1998, 54-84.
14. Spanos GA, Wrosltad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural & Food Chemistry. 1990; 38:1565-1571.
15. Liu X, Dong M, Chen X, Jiang M, Lv X, Yan G. Antioxidant activity and phenolics of endophytic *Xylaria* sp. From *Ginkgo biloba*. Food Chemistry. 2007; 105:548-554.
16. Stahl E. Thin Layer Chromatography, 2nd ed., Springer Pvt. Ltd., New Delhi: 2005, p85.
17. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischaemic heart disease in a Welsh population of men - the Caerphilly study. American Journal of Clinical Nutrition. 1997; 65:1489-1494.
18. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartfield PW, *et al*. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry. 1998; 46:1887-1892.
19. D'Cunha M, Sridhar KR. L-canavanine and L-arginine in two wild legumes of the genus *Canavalia*. Institute of Integrative Omics and Applied Biotechnology. Journal. 2010; 1:29-33.
20. Kruse PR, McCoy TA. The competitive effect of canavanine on utilization of arginine in growth of Walker carcinosarcoma 256 cells in vitro. Cancer Research. 1958; 18:279-282.
21. Green MH, Brooks TL, Mendelsohn L, Howell SB. Antitumor activity of L-canavanine against L1210 murine leukemia. Cancer Research. 1980; 40:535-537.
22. Thomas FA, Rosenthal GA, Gold DV, Dickey K. Growth inhibition of a rat colon tumor by L- canavanine. Cancer Research. 1986; 46:2898-2903.
23. Mattei E, Damasi D, Mileo AM, Delpino A, Ferrini U. Stress response, survival and enhancement of heat sensitivity in a human melanoma cell line treated with L-canavanine. Anticancer Research. 1992; 12:757-62.
24. Swaffar DS, Ang CY, Desai PB, Rosenthal GA. Inhibition of the growth of human pancreatic cancer cells by the arginine antimetabolite L-canavanine. Cancer Research. 1994; 54:6045-6048.

25. Saraswathi K, Mahalakshmi B, Rajesh V, Arumugam P. *In vitro* Evaluation of Antioxidant and Antimicrobial Potential of leaves of *Atalantia racemosa* Wight ex Hook. Int J Pharma Res Health Sci. 2017; 5(6):2031-37.
26. Pisoschi AM, Negulescu GP. Methods for Total Antioxidant Activity Determination: A Review. Biochem & Anal Biochem. 2011; 1:106.
27. Litescu SC, Sandra AV, Eremia SAV, Diaconu M, Tache A. Biosensors Applications on Assessment of Reactive Oxygen Species and Antioxidants. Environmental Biosensors. In Tech Rijeka Croatia, 2011.
28. Pellegrini N, Serafini S, Del Rio SD, Bianchi M. Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different *in vitro* assays. Mol Nutr Food Res. 2006; 50:1030-1038.
29. Hu FB. Plant-based foods and prevention of cardiovascular disease: an overview. Am J Clin Nutr. 2003; 78(3):544-551.
30. McCullough ML, Robertson AS, Chao A, Jacobs EJ, Stampfer MJ. A prospective study of whole grains, fruits, vegetables and colon cancer risk. Cancer Cause Control, 2003; 14:959-970.
31. Campanella L, Martini E, Rita E, Tomassetti M. Antioxidant capacity of dry vegetal extracts checked by voltammetric method. J Food Agric Environ. 2006; 4:135-144.
32. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J. 2013; 21:143-152.

HOW TO CITE THIS ARTICLE

Saraswathi K, Rajesh V, Saranya R, Arumugam P. GC-MS, phytochemical analysis and *in vitro* antioxidant activities of leaves of *Canavalia cathartica* Thouars. J Phytopharmacol 2018; 7(3):263-269.