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 8; Issue 03 (D); March 2019; Page No.17822-17827
DOI: http://dx.doi.org/10.24327/ijcar.2019.17827.3395

Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF ALKALOIDS AND FLAVONOIDS CONTENTS OF METHANOLIC EXTRACTS OF DODONAEA VISCOSA (L) JACQ AND ACALYPHA INDICAL

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A R T I C L E I N F O

Article History:
Received 06th December, 2018
Received in revised form 14th January, 2019
Accepted 23rd February, 2019
Published online 28th March, 2019

Key words:
Phytochemicals, Alkaloids, Flavonoids, DPPH assay.

A B S T R A C T

The present study aimed to establish the phytochemical profile of methanol, ethanol, chloroform and water extract of leaves of Dodoneae viscosa (L) Jacq and Acalypha indica L. The research study revealed the presence of alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phenols, saponins, tanins, carbohydrates and oil respectively and the methanolic extract of both the plant sample contains most of the phytochemicals tested compared to other solvents. From the methanolic extract of both the plant sample crude alkaloids and crude flavonoids were extracted and their antioxidant activity were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and H2O2 method. The methanolic extract of Dodoneae viscosa (L) Jacq was found to have higher amount of crude alkaloids content while the crude flavonoids content was more in Acalypha indica L. The antioxidant activity study revealed that the crude alkaloids of Dodoneae viscosa (L) Jacq and crude flavonoids of Acalypha indica L were found to have more free radical scavenging activity and it could explain and justify some of their uses in traditional medicine and they need further exploration for their use in modern system of medicines.

INTRODUCTION

Since time immemorial, plant have supported humans not just for survival but have been source for phytomedicines enabling a healthy living. Different plants provide different medicinal properties. This can be obtained from barks, roots, fruits, flowers and also seeds. [1]. *Dodonaea viscosa* (L) Jacq belongs to the family *Sapindaceae*. This plant grows abundantly in continents of Australia, Asia and South America. This plant is either dioecious or monoecious in nature growing up to 7 meters tall with angled branches and black coloured twigs. This plant showcases variety of phytochemicals such as terpenoids, saponins, flavonoids and a several phenolic components. It possesses several applications in the research of drugs and therapeutics. It has been used since olden times for treatment of different health disorders like sore throats, fever, cold, relieve itching, digestive system disorders, fevers swellings and acts as an antispasmodic agent [2]. The seeds provide relief in symptoms of malaria while the stems help cure rheumatism. It can be utilized in lotion form to treat sprains, bruises, burns and wounds [3]. *Acalypha indica L* another medicinally important plant extensively studied grows in the deciduous and mixed-monsoon forests regions of India. It belongs to family *Euphorbiaceae* under subfamily

Acalyphoideae. It possesses several useful therapeutic properties like antitussive, hepatoprotective, antifungal, antibacterial, anti-tuberculosis and anti-inflammatory [4]. The roots are known to be astringent, used in cases of fever and can be purgative with its leaves diminishing the mutagenicity in E. coli. It finds several applications for chilblains, rheumatism, facial paralysis, anti-asthamatic, insect bites, jaundice, piles, swelling, and externally skin eruptions [5]. Plants used in clinical drug formulations are thus extensively studied to understand their primary metabolism products like the carbohydrates, proteins, nucleic acids and amino acids. Apart from these several secondary metabolites like phytochemicals present in plants are frequently analysed such as alkaloids, flavonoids, tannins, steroids, terpenoids, etc. [6].

In the previous of our study the leaves of *Dodonaea viscosa (L) Jacq* and *Acalypha indica L* were used to treat cancer has been documented [7]. The phytochemicals present in these plants are the source of antioxidants and responsible to cure various human diseases have been well known. So the aim of the present study is to establish the preliminary

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phytochemicals profile, quantitative analyses and free radical scavenging activity of phytochemicals present in these medicinally important plants of Sira taluk, Tumkur district, Karnataka state, India.

MATERIALS AND METHODS

Collection and Identification of Plants

The plants were collected from the different region of Sira taluk, Tumkur district, Karnataka, India (from their natural habitat with acceptable bio-conservation methods) have been identified and authenticated by Regional Ayurveda Research Institute for Metabolic Disorders Bangalore as Dodonaea viscosa (L) Jacq (Ref: RRCBI-13062) and Acalypha indica L (Ref: RRCBI-15581), belongs to the family Sapindaceae and Euphorbiaceae respectively [8].

Plant Material

The leaves of Dodonaea viscosa (L) Jacq and Acalypha indica L were washed thoroughly under running tap water followed by distilled water and then air dried under shade at room temperature for one week. The dried leaves were powdered with the help of porcelain mortar and pestle to increase the surface area for absorption of the solvents (Harborne 1973), stored in separate containers in moisture free environment and used for further analysis.

Preparation of Extract

Four solvents were selected for the process of extraction on account of their polarity, namely Methanol, Ethanol, Chloroform, and Water. 30 gm of dried leaves powder of each plant were taken separately and process of extraction using Soxhlet apparatus was performed out in a 250 mL of each solvent separately. The extraction process was time framed for complete 48 hrs after which the solvent mixture was concentrated at a temperature not exceeding 40^{o}C using a rotary evaporator and stored at 4^{0}C [9]. The percentage yield and other physical properties were observed [10].

Preliminary Phytochemical Screening

Different solvent extract of each plant were subjected to chemical test for different phytochemicals viz. Alkaloids, Flavonoids, Steroids, Terpenoids, Anthraquinones, Phenols, Saponins, Tanins, Carbohydrates and Oil by using standard procedures [11-26].

Quantitative Analysis of Phytochemicals

Total Alkaloid estimation by Using Harborne (1973) Method.
5 g of the methanolic extract of each plant sample was weighed separately into a 250 mL capacity beaker and added 200 mL of 10% acetic acid in ethanol then covered the beaker to check evaporations of solvent and allowed to stand for 4 hours. This was filtered and the extracts were concentrated on water bath to ¼ of original volume. Then conc. ammonium hydroxide solution was added drop wise into concentrated extracts until the precipitation was completed. The solution was allowed to settle the precipitate and filtered. The filtered precipitate washed with dil. ammonium hydroxide and then again filtered. This precipitate residue is alkaloid which was dried and weighed.

Weight of total alkaloids: $\frac{w2-w1}{w3}g$

% Yields of Alkaloid: $\frac{w2-w1}{w3}X100$

Where, W1 = weight of crucible, W2 = weight of crucible with alkaloids, W3 = initial weight of plant sample taken for estimation [27].
Flavonoid Determination by the Method of Boham and Kocipai- Abyazan (1994**)**

10 g of the methanolic extract of each plant sample was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [28].

Weight of total flavonoids: $\frac{w2-w1}{w3}g$

% Yields of flavonoids: $\frac{w2-w1}{w3}X100$

Where, W1 = weight of crucible, W2 = weight of crucible with flavonoids, W3 = initial weight of plant sample taken for estimation.

DPPH radical Scavenging assay

Free radical scavenging activity of ascorbic acid and crude alkaloids and crude flavonoids of methanolic extract of both Dodonaea viscosa (L) Jacq and Acalypha indica L were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) using the modified method [29]. Briefly, 1 mL of different concentrations of ascorbic acid (50-250 µg/mL) and crude alkaloid and flavonoid sample solution in methanol (250, 200, 150, 100, and 50 µg/mL) was mixed with 2 mL of a freshly prepared solution of 0.1mM (0.04 mg/mL) DPPH in methanol. The mixture was allowed to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm by using spectrophotometer (UV-VS Shimadzu 1700). The all experiments were performed thrice and the results were averaged. The various concentrations of crude samples were prepared by dilution method [30]. The IC_{50} value is the concentration of sample required to scavenge the 50% of initial concentration of DPPH radical and was calculated from the plotted graph of percentage inhibition against the various concentrations of the crude samples.

The percentage inhibition of DPPH radical was calculated as follows:

% inhibition = [$Abs_{control} - Abs_{sample}$/ $Abs_{control}$] X 100
Where $Abs_{control}$ is the absorbance of DPPH radical + methanol and Abs_{sample} is the absorbance of DPPH + crude samples/ascorbic acid.

Hydrogen Peroxide Radical Scavenging Activity

The crude alkaloids and crude flavonoids of methanolic extract of both the plants were tested to determine the hydrogen peroxide scavenging activity along with standard by Ruch *et al* [31]. The hydrogen peroxide solution (40mM) was prepared in 0.1M phosphate buffer (p^H 7.4) and the concentration of H_2O_2 was determined at 230nm by spectrophotometer (UV-VS Shimadzu 1700). The different concentrations (20-120 µg/mL) of crude extract and standard was added to 0.6mL of H_2O_2 solution. After 10 min the decrease in absorbance was measured at 230nm against blank solution containing phosphate buffer solution without hydrogen peroxide. All the tests were performed in triplicates. The percentage of H_2O_2

scavenged by the extract and standard was calculated as follows:

% of H_2O_2 scavenged = [Abs $_{control}$ – Abs $_{sample}$/ Abs $_{control}$] X 100

The IC_{50} value was determined from the plotted graph of % inhibition vs various concentration of extract/standard.

RESULT

The Percentage yield of the Extract

The dried leaf powder of the plants was extracted with different solvents such as water, methanol, ethanol and chloroform and the percentage yield of the extract was determined. The percentage yield of dodonaea viscosa *(L) Jacq* was water (2.2%w/w) > chloroform (2.0%w/w) > ethanol (1.3%w/w) > methanol (0.5% w/w) and for acalypha *indica L* methanol (2.3%w/w) > water (2.1%w/w) > chloroform (1.5%w/w) > ethanol (0.9%w/w) respectively. From the different solvent extract; the water extract of dodonaea *viscosa (L) Jacq* (2.2% w/w) and methanol extract of acalypha *indica L* (2.3%w/w) was found to have high percentage yield compared to other solvent extract (Table 1).

Preliminary Phytochemical study

In order to establish the preliminary phytochemical profile of plants, the different solvent extract of these plants were subjected to various chemical tests and it revealed the presence of alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phenols, saponins, tannins, carbohydrates and oil. The methanolic extract of dodonaea *viscosa (L) Jacq* showed the presence of most of the phytochemicals tested except steroids where as terpenoids and saponins were absent in methanol extract of acalypha *indica L* (Table 2).

Quantitative Analysis

The crude alkaloids and crude flavonoids content of methanolic extract of these plants were determined by standard procedure. The result showed that the amount of crude flavonoids (1.3%w/w) > crude alkaloids (1.25%w/w) in dodonaea *viscosa (L) Jacq* and in Acalypha *indica L* the crude flavonoids (2.37%w/w) > crude alkaloids (0.5%w/w) content (Table3).

Free Radical Scavenging Activity by DPPH Method

The free radical scavenging activity of different concentration of crude alkaloids and crude flavonoids of both the plants were measured using DPPH method, the percentage inhibition and IC_{50} value was calculated and the results were compared with standard ascorbic acid. The DPPH free radical scavenging activity study results showed that at 250 µg/mL concentration of crude alkaloids of dodonaea *viscosa (L) Jacq* had 93.86 percentage inhibition with IC_{50} value of 134.6 µg/mL and at the same concentration, the crude flavonoids showed 65.72 percentage inhibition with IC_{50} value of 220.64 µg/mL. The percentage inhibition of crude flavonoids of acalypha *indica L* at 250 µg/mL were found to have 92.22% of inhibition with IC_{50} value of 133.06 µg/mL where as crude alkalids showed 70.65% of inhibition with IC_{50} value of 220µg/mL respectively (Table 4).

Free Radical Scavenging Activity by H_2o_2 Method

The results of hydrogen peroxide scavenged assay revealed that at 120 µg/mL concentration the crude alkaloids of dodonaea *viscosa (L) Jacq* showed the percentage inhibition of 95.49% with IC_{50} value of 27.38 µg/mL where as 57.13% with 78.94µg/mL of acalypha *indica L*. The crude flavonoids of acalypha *indica L* were found to have 94.29% of inhibition with IC_{50} value of 41.9 µg/mL and 76.21% of inhibition with IC_{50} value of 59.32 µg/mL were found in dodonaea viscosa *(L) Jacq* (Table 5).

Table 1 The % yield of different solvent extract of Dodonaea viscosa (L) Jacq and Acalypha indica L leaves

Properties	Dodonaea viscosa (L) Jacq				Acalypha indica L			
	Water	Methanol	Chloroform	Ethanol	Water	Methanol	Chloroform	Ethanol
Surface	Dry	Oily	Oily	Oily	Dry	Oily	Oily	Oily
Colour	Brown	Brown	Yellowish brown	Green	Brown	Black	Yellowish brown	Yellowish brown
%Yield (w/w)	2.2	0.5	2.0	1.3	2.1	2.3	1.5	0.9

Table 2 Preliminary phytochemical profile of Dodonaea viscosa (L) Jacq and Acalypha indica L leaves.

Sample	Alkaloids	Flavonoids	Steroids	Terpenoids	Anthraquinones	Phenols	Saponins	Tanins	Carbohydrates	Oil
S1W	+	--	--	+	+	+	+	--	--	--
S1M	+	+	--	+	+	+	+	+	+	+
S1C	--	+	+	--	--	--	--	--	+	+
S1E	+	+	+	--	+	+	--	+	--	+
S2W	+	--	--	--	+	--	+	+	--	--
S2M	+	+	+	--	+	+	--	+	+	+
S2C	+	+	--	--	--	+	--	--	--	+
S2E	--	+	--	+	--	+		+	--	+

S1= Dodonaea viscosa (L) Jacq, S2= Acalypha indica L, W=Water, M=Methanol, C= chloroform and E= Ethanol.

Table 3 Quantitative analysis of crude alkaloids and crude flavonoids

Sample	Alkaloids(%w/w)		Mean	SD	Flavonoids(%w/w)		Mean	SD
	1	2			1	2		
S1M	1.2	1.3	1.25	±0.07	1.4	1.2	1.3	±0.14
S2M	0.5	0.5	0.5	±0.00	2.3	2.44	2.37	±0.10

S1= Dodonaea viscosa (L) Jacq, S2= Acalypha indica L and M=Methanol extract

Table 4 Percentage Inhibition and IC_{50} value by DPPH Assay.

Plants	Crude Alkaloids		Crude Flavonoids		Standard Ascorbic acid	
	% Inhibition	IC_{50} (µg/mL)	% Inhibition	IC_{50} (µg/mL)	% Inhibition	IC_{50} (µg/mL)
D.*viscosa*	93.86	134.6	65.72	220.64	95.89	75.86
A.*indica*	70.65	220	92.22	133.06		

Table 5 Percentage Inhibition and IC_{50} values by H_2O_2 method

Plants	Crude Alkaloids		Crude Flavonoids		Standard Ascorbic acid	
	% Inhibition	IC_{50} (µg/mL)	% Inhibition	IC_{50} (µg/mL)	% Inhibition	IC_{50} (µg/mL)
D.*viscosa*	95.49	27.38	76.21	59.32	97.2357	31.2
A.*indica*	57.13	78.94	94.29	41.9		

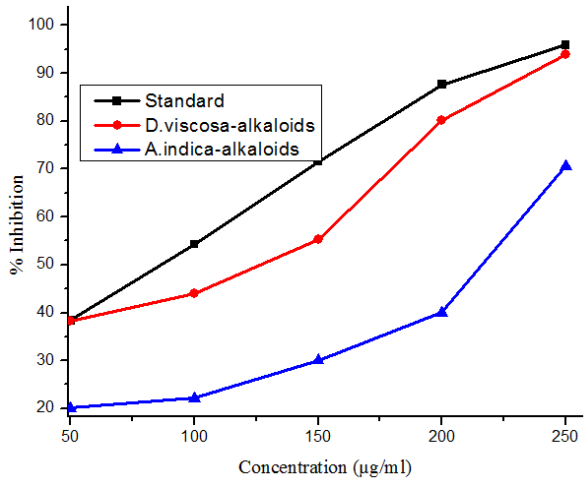

Figure 1 Comparison graph of Crude Alkaloids of plants by DPPH method

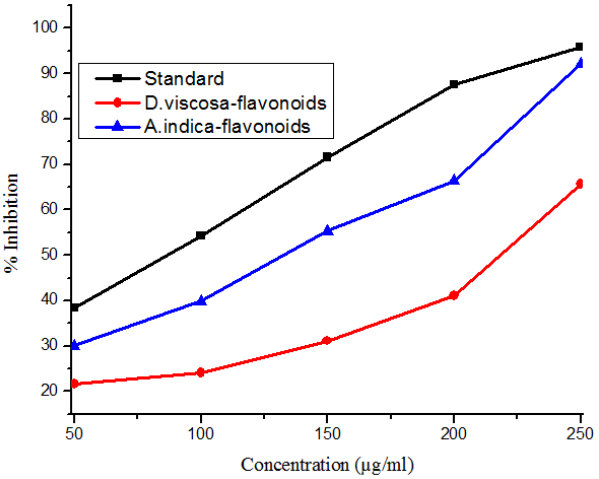

Figure 2 Comparison graph of Crude Flavonoids of plants by DPPH method

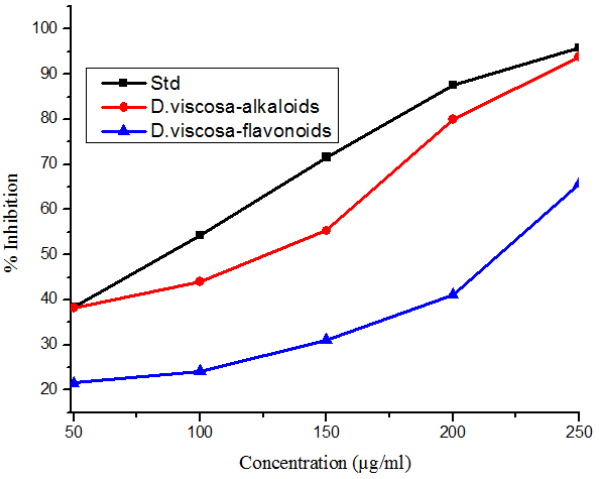

Figure 3 comparison graph of crude alkaloids and crude flavonoids of dodonaea viscosa (l) jacq by dpph method

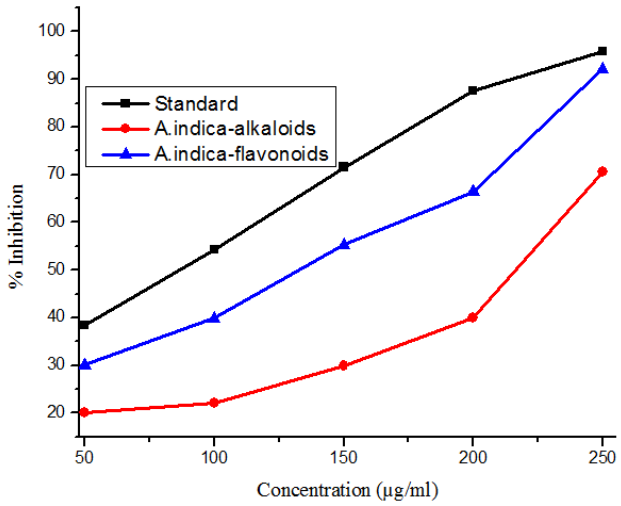

Figure 4 Comparison graph of Crude Alkaloids and Crude Flavonoids of Acalypha indica L by DPPH method

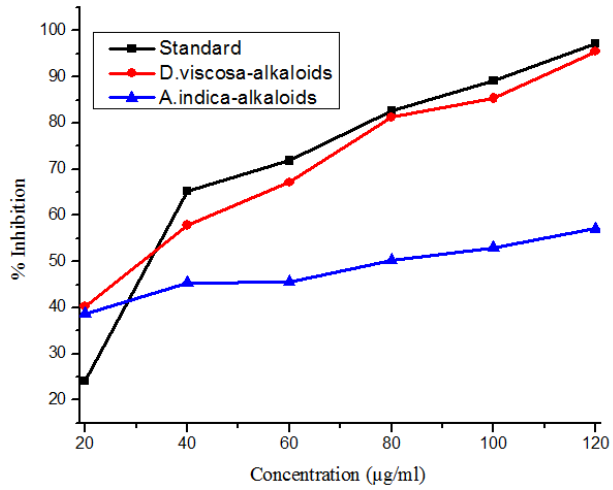

Figure 5 Comparison graph of Crude Alkaloids of plants by H_2O_2 method

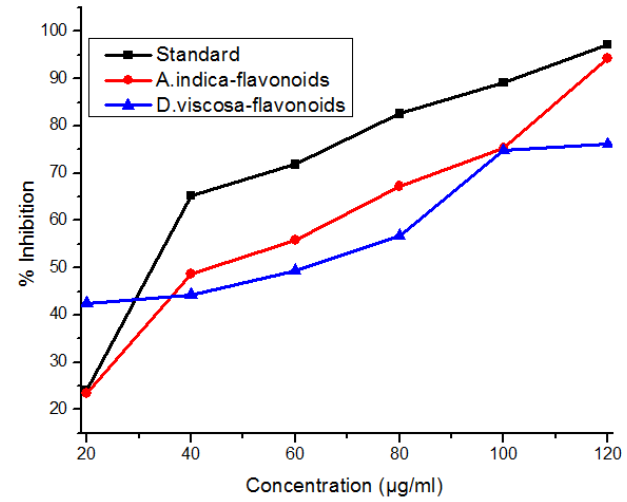

Figure 6 Comparison graph of Crude Flavonoids of plants by H_2O_2 method

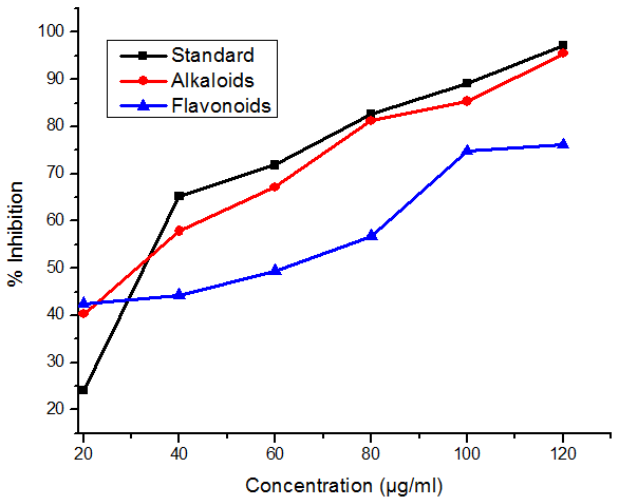

Figure 7 Comparison graph of Crude Alkaloids and crude flavonoids of D. viscosa by H_2O_2 method

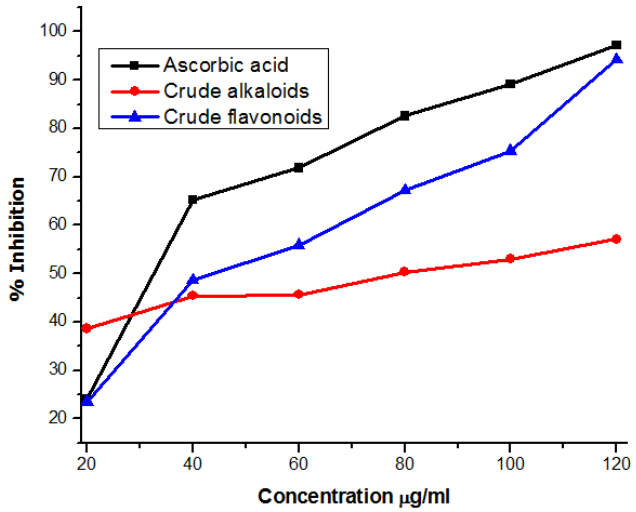

Figure 8 Comparison graph of Crude Alkaloids and crude flavonoids of A.indica by H_2O_2 method

DISCUSSION

The present work is conducted on methanol, ethanol, chloroform and water extract of *dodonaea viscosa (L) Jacq* and *acalypha indica L* plant leaf to establish the phytochemical profile and quantification further the methanolic extract of crude alkaloids and crude flavonoids were evaluated for free radical scavenging activity study by DPPH and H_2O_2 method. Phytochemicals analysis confirmed several phytochemicals present in these extracts such as flavonoids, alkaloids, phenols, terpenoids, tannins, steroids, oil, saponins, and anthraquinones. The antioxidant activity of crude alkaloids and crude flavonoids of methanolic extract of these plants has been performed in in-vitro model and compared with standard ascorbic acid. DPPH is free radical that can readily bind with antioxidant compounds by accepting a hydrogen atom and is noted by the change of colour. This colour change into deep violet is estimated calorimetrically at 517 nm. Higher the intensity of colour higher is the inhibition of DPPH activity. The DPPH scavenging activity of ascorbic acid, crude alkaloids of both the plants were compared, the result showed that the percentage inhibition of ascorbic acid (95.89%) > D. viscosa (93.86%) > acalypha indica (70.65%) while percentage inhibition of the crude flavonoids were found in the order; acalypha indica (92.22%) > D. viscosa (65.72%). The hydrogen peroxide scavenging capacity was also compared. The percentage scavenging activity of crude alkaloids of D. viscosa (95.49%) > A. indica (57.13%) and crude flavonoids of A. indica (94.29%) > D. viscosa (76.21%).

CONCLUSION

This appears to be the first report on the study of phytochemicals and antioxidant activity of dodonaea *viscosa (L) Jacq* and acalypha *indica L* plants of Sira Taluk, Tumkur District, Karnataka State, India. The results of our study showed that, the methanolic extract of leaves of these plants are good source of phytochemicals and comapared to ethanol, chloroform and water; methanol was the better solvent of their extraction. Further, the crude alkaloids of dodonaea *viscosa (L) Jacq* and crude flavonoids of acalypha *indica L* showed potent free radical scavenging activity both in DPPH and H_2O_2 method. These plants possesses pharmaceutically important phytochemicals and their free radical scavenging activity is attributed to the higher amount of alkaloids and flavonoids content and it could justify the use of these plants to treat cancer by the local people of Sira taluk, Tumkur district, Karnataka state, India.

Conflict of Interest Statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to thank Tumkur University, Tumkur for the necessary help.

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How to cite this article:

Shivakumara S.L *et al* (2019) 'Preliminary Phytochemical Screening and Antioxidant Activity of Alkaloids and Flavonoids Contents of Methanolic Extracts of Dodonaea Viscosa (l) Jacq and Acalypha Indical', *International Journal of Current Advanced Research,* 08(03), pp.17822-17827. DOI: http://dx.doi.org/10.24327/ijcar.2019.17827.3395

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