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Phytochemical Profiling and *in vitro* Antioxidant Activity of Leafless Mistletoe *Viscum articulatum* Burm.f. by DPPH Assay

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ABSTRACT

Background: *Viscum articulatum* Burm.f. is a hemiparasitic plant belongs to family Viscaceae, which having various traditional medicinal uses. The present work was attempted to determine phytoconstituents and *in vitro* antioxidant activity of *V. articulatum* by using DPPH scavenging assay to evaluate their potential as to elucidate pharmacological actions.

Methods: The dried plant material powdered administered to soxhlet extraction with petroleum ether, benzene, chloroform, acetone, ethanol and distilled water respectively for 18 h. The condensed extracts were tested for qualitative assessment of phytochemicals. *In vitro*, antioxidant activity was conducted via DPPH radical scavenging assay. Antioxidant activity was assessed with acetone and ethanolic crude extract to evaluate the free radical scavenging activity (Inhibition (%)/ Scavenging) of *V. articulatum* at different concentrations of stock solution likes 50, 100, 150, 200, and 250 µg/ml.

Results: Phytochemical investigation prominently reveal the presence of phytoconstituents like carbohydrates, cardiac glycosides, proteins, alkaloids, fats, saponin, coumarins, flavonoids, tannins, phenolics, steroids and quinone, which have been responsible for various biological activities. The IC_{50} value of acetone and ethanol extract of the studied plant was found to be 9.4 and 8.9 respectively, which was comparable with standard ascorbic acid (IC_{50} = 5.4).

Conclusion: The results suggest that *V. articulatum* exhibits the excellent potential of antioxidant activity that may be beneficial for its medicinal values.

Key-words: Antioxidant activity, DPPH assay, *in vitro*, Phytochemical, *V. articulatum*

INTRODUCTION

Since ancient times, plants are a vital source of bioactive compounds with antioxidant properties [1]. An antioxidant is known as the substance that inhibits a target molecule's oxidative damage [2,3]. The essential substances that can protect the body from damage caused by free radical oxidative stress are antioxidants [4]. An antioxidant's key particularity is its ability to trap free radicals. Many ailments, including cancer, Parkinson's disease, Alzheimer's disease, cardiovascular disorders and neurological disorders, can be caused [5].

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Often responsible for human ageing are reactive oxygen species [6]. The oxidative processes that lead to degenerative diseases are blocked by antioxidant substances such as phenolic acids, polyphenols and flavonoids that scavenge free radicals [7,8]. They are occurring naturally and through synthetic chemical processes. There is now a growing trend to substitute synthetic antioxidants with natural antioxidants because safety is a concern [9]. The epidemiological studies have revealed that the utilization of natural antioxidants is related to a lower risk of cardiovascular diseases and cancer. The natural antioxidants are isolated products of plant origin [10]. So, herbal plants considered as an excellent antioxidant since long years ago.

V. articulatum Burm.f. is a hemiparasitic plant belongs to family Viscaceae commonly parasite on the stem of *Diospyrous melanoxylon* Roxb. and *Boswellia serrata* Roxb. Also, it is rarely epiparasites on the stem of

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Dendropthae falcata (L.f.) Ett. This plant is used traditionally for anti-cancerous properties, bone fractures, febrifuge, inflammations, lumber muscles strain, psoriasis, rheumatic swelling and urinary tract infection.

Therefore, the present work was attempted to evaluate phytoconstituents and *in vitro* antioxidant activity of *V. articulatum* plant by using DPPH scavenging assay to assess their ability to elucidate pharmacological activities.

Fig. 1: Plant of *V. articulatum* Burm.f.

MATERIALS AND METHODS

The plant materials under study, i.e. *V. articulatum* Burm. f. was collected during the period of flowering and fruiting from Melghat forest region district Amravati. The herbarium specimen was prepared, identified with the help of standard floras [11-13] and the study was carried out in December 2018 at the Department of Botany, Bharti Mahavidyalaya Arni, district Yavatmal, Maharashtra, India.

Phytochemical analysis- The collecting plant material was washed and shade dried. The dried plant material is powdered by using grinder mixer and in the order of increasing polarity of solvents, it was subjected to soxhlet extractions with petroleum ether, benzene, chloroform, acetone, ethanol and distilled water for 18 hrs, respectively. The condensed extracts were used for qualitative assessment of phytochemicals. It involved the examination of various classes of compounds. A qualitative chemical test followed by the method chosen for the identification of different phytoconstituents to provide a general idea about the existence of constituents present in crude drugs [14-17]. The extracts were analyzed for the presence of phytochemicals like carbohydrates, cardiac glycosides, alkaloids, flavonoids, tannin, phenolics, steroids, coumarins and saponin.

***In Vitro* Antioxidant activity-** The DPPH radical scavenging assay carried out the antioxidant activity of *V. articulatum* [18] in acetone as well as ethanol extract. The percentage % of scavenging (inhibition) is calculated in triplicates.

Preparation of acetone and ethanolic extract- Total 100 gm dried plant powder extracted with acetone and ethanol in increasing order of nature of polarity of solvent by Soxhlet apparatus. By using a rotary flash evaporator, the extracts were concentrated then dried in a desiccator with the residue.

DPPH Radical Scavenging Assay (2,2-diphenyl-1-Picrylhydrazyl)- Based on the radical scavenging effect of the stable DPPH radical, the anti-oxidant activity of *V. articulatum* acetone and ethanolic extracts were carried out. DPPH is a stable free radical, which can accept hydrogen radical electrons to become a stable diamagnetic molecule. DPPH shows a potent absorption band at 517 nm. Antioxidant reacts with DPPH, which is a stable free radical and reduces DPPH to DPPH–H (2, 2-diphenyl-1-Picrylhydrazine). The absorption decreases in the degree of discolouration from the DPPH radical (Purple) to DPPH–H (Yellow) indicates the scavenging potential of the antioxidant compound or extracts in terms of the ability to donate hydrogen [19-22].

Preparation of *V. articulatum* Burm.f stock solution- The 0.1 mM DPPH solution was prepared with ethanol. 1 ml of solution was then added to 3ml of ethanol extract at varying concentrations (50, 100, 150, 200 and 250 µg/ml). Only those extracts which are emulsifiable in ethanol are used here and their variable concentrations were prepared using the method of dilution. The mixture was then shaken vigorously and allowed to stand for 30 minutes at room temperature and thereafter absorbance was recorded at 517 nm by using a spectrophotometer (Systronics UV-VIS Spectrophotometer) [23]. As a standard, Ascorbic acid was used for comparison by making the same dilutions as extracts of *V. articulatum* stocks (50, 100, 150, 200, and 250 µg/ml). A mixture of 1 ml of ethanol and 1 ml DPPH was used as a control and a formula was used to measure the percentage (%) of inhibition/scavenging as-

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$$\text{Inhibition (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where, $A_{Control}$= Control Absorbance, A_{Sample}= Sample Absorbance

The graph was plotted with a concentration of µg/ml on X-axis and a percentage of inhibition on Y-axis and the IC_{50} value of the sample, which was the sample concentration needed to inhibit 50% of the free radical DPPH, was determined using inhibition curve [24].

RESULTS

Phytochemical profiling- The qualitative phytochemical assessment of *V. articulatum* was performed on six different extracts, i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water, which revealed that phytoconstituents such as carbohydrates, cardiac glycosides, proteins, alkaloids, fats, saponins, coumarins, flavonoids, tannins, phenolics, steroids and quinine were prominently present. In all the extracts, however, anthraquinone glycoside was completely absent. The presence of carbohydrates, cardiac glycosides, proteins, alkaloids, saponins, flavonoids, fats, tannins and phenolics have been demonstrated in the ethanol extract of *V. articulatum*. Also, the presence of all compounds except steroids and quinone was shown by water extract. The existence of a minimum number of phytoconstituents was demonstrated by petroleum ether, benzene and chloroform extracts. The presence of alkaloids and proteins was only demonstrated by acetone, ethanol and water extract (Table 1).

Table 1: Qualitative phytochemical screening of *V. articulatum* Burm.f.

S.No.	Constituents	Chemical Tests	Extracts					
			P. E.	B	C	A	E	W
1	Alkaloids	Hager's Test	+	-	-	+	+	+
		Mayer's Test	-	-	-	+	+	+
		Wagner's Test	-	-	-	+	+	+
		Dragendroff's Test	-	-	+	+	+	+
2	Carbohydrates & Glycosides	Fehling's Test	-	-	+	-	-	-
		Benedict's test	-	-	+	+	+	+
		Molisch's Test	-	+	+	+	+	+
3	Steroids	Salkowski Test	+	+	-	+	+	-
4	Saponin	Foam Test	-	+	+	-	+	+
5	Phenolics & Tannin	$FeCl_3$ Sol. Test	-	-	-	+	+	+
		Lead Acetate Test	-	-	-	+	+	+
6	Fixed oil & Fats	Spot Test	-	-	+	+	+	+
7	Proteins	Biuret Test	-	-	-	-	+	+
		Million's Test	-	-	-	+	+	+
8	Anthraquinone glycosides	Borntrager's Test	-	-	-	-	-	-
9	Cardiac glycosides	Keller-Killiani Test	+	+	+	-	+	+
10	Flavonoids	Shinoda Test	-	+	-	-	+	+
		Lead Acetate Test	-	-	+	+	+	+
11	Quinone		-	-	-	+	+	-
12	Coumarins		+	+	-	-	-	+

'+' = Present and '-' = Absent
P.E.= Petroleum ether, B= Benzene, C= Chloroform, A= Acetone, E= Ethanol, W= Water extract respectively

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***In-vitro* Antioxidant activity-** DPPH radical scavenging activity is primarily one of the methods for *in vitro* antioxidant screening of plant extracts [1]. Acetone and ethanolic extracts of *V. articulatum* were studied for antioxidant potential of different concentration of stock solutions viz. 50,100, 150, 200 and 250 µg/ml. As a standard, ascorbic acid was used. The antioxidant characteristics depend on the value of IC_{50}. Acetone and Ethanol extracts of *V. articulatum* showed increased in DPPH scavenging activity with a corresponding increase in its concentration (Table 2 & 3, Fig. 2 & 3). Ethanol extract of this plant showed good antioxidant activity with an IC_{50} value of ascorbic acid. Fig. 2 and 3 shows the comparative data of DPPH radical scavenging activity of different extracts with standard ascorbic acid, respectively. The IC_{50} value was the concentration of the sample required to inhibit 50% of the free radicals present in the system. Conversely, the IC_{50} value was connected to the crude extract's antioxidant activity. In this result, IC_{50} value was lower and the antioxidant activity was the more. Based on the result obtained, ethanolic extract of this plant showed good antioxidant activity (i.e. IC_{50}= 8.9) than that of the acetone extract (i.e. IC_{50}= 9.4) as compared to standard ascorbic acid (i.e. IC_{50}= 5.4) as shown in Table 4 and Fig. 4, 5 and 6.

Table 2: Antioxidant activity of *V. articulatum* Burm.f. acetone extract

S. No.	Concentration (µg/ml)	Inhibition (%)	
		Acetone extract	Ascorbic acid
1	50	55.40 ± 0.46	60.32 ± 0.05
2	100	62.78 ± 0.60	68.33 ± 0.16
3	150	65.44 ± 0.51	80.21 ± 0.04
4	200	75.54 ± 0.13	96.90 ± 0.12
5	250	81.13 ± 0.31	97.25 ± 0.1

Values represent mean ± SD (*n*=3)

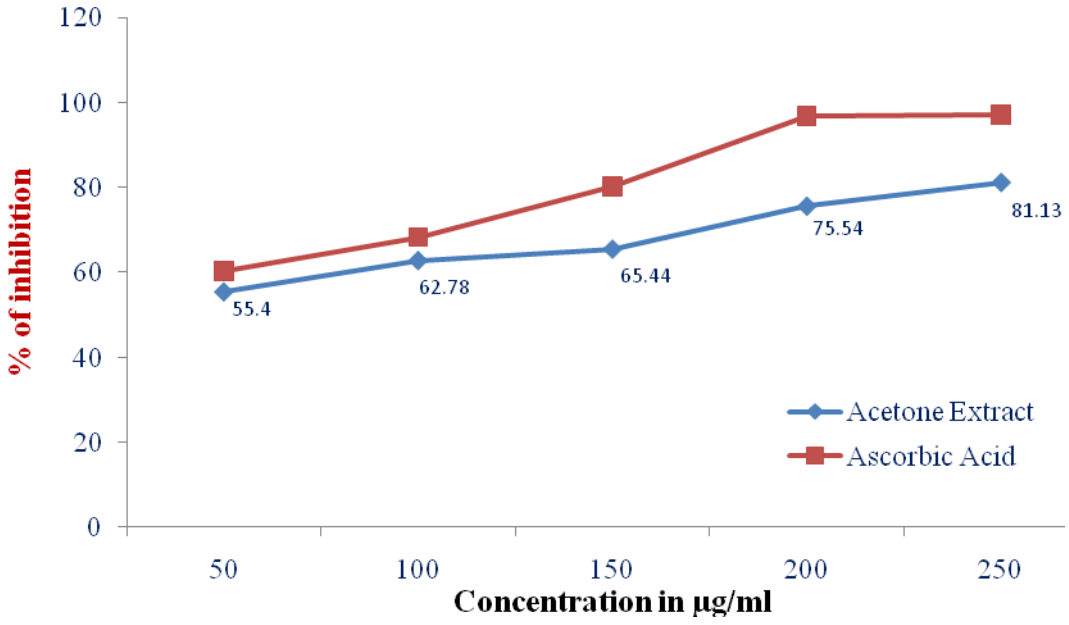

Fig. 2: Antioxidant activity of *V. articulatum* Burm.f acetone extract

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Table 3: Antioxidant activity of *V. articulatum* Burm.f. ethanol extract

S. No.	Concentration (µg/ml)	Inhibition (%)	
		Ethanol extract	Ascorbic acid
1	50	58.38 ± 0.47	60.32 ± 0.05
2	100	60.40 ± 0.40	68.33 ± 0.16
3	150	71.31 ± 0.30	80.21 ± 0.04
4	200	78.47 ± 0.33	96.90 ± 0.12
5	250	86.62 ± 0.34	97.25 ± 0.1

Values represent mean ± SD (*n*=3)

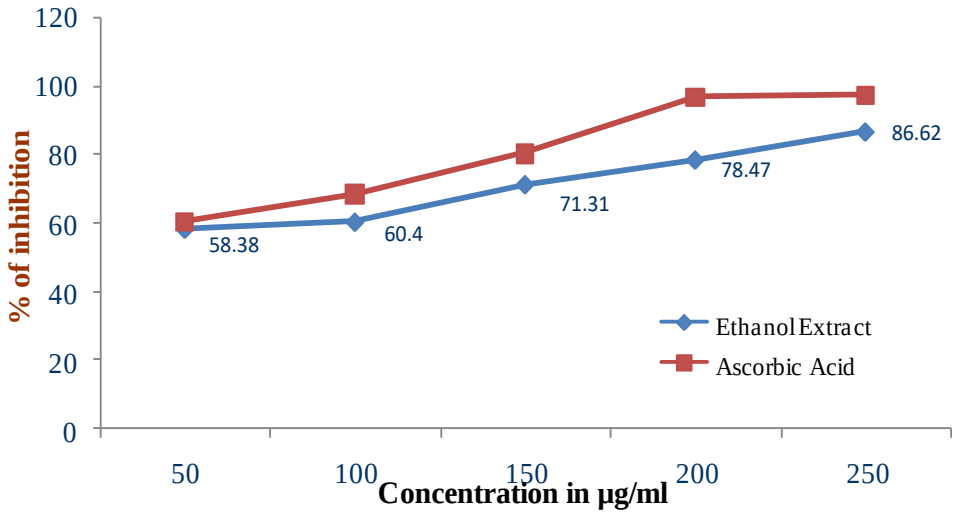

Fig. 3: Antioxidant activity of *V. articulatum* Burm.f ethanol extract

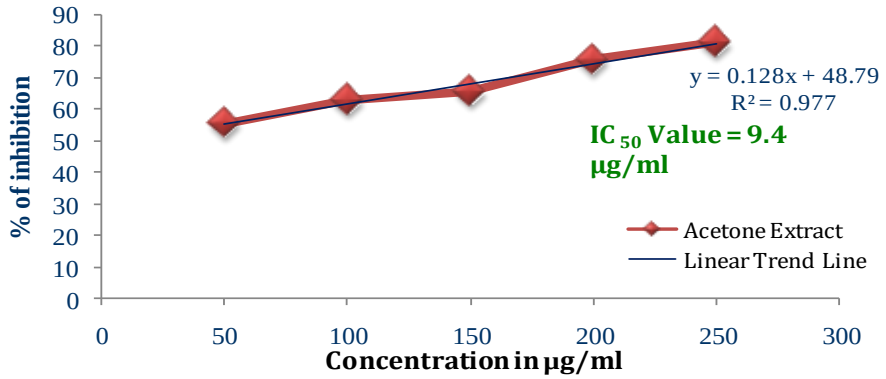

Fig. 4: IC_{50} calculation of *V. articulatum* Burm.f in acetone extract

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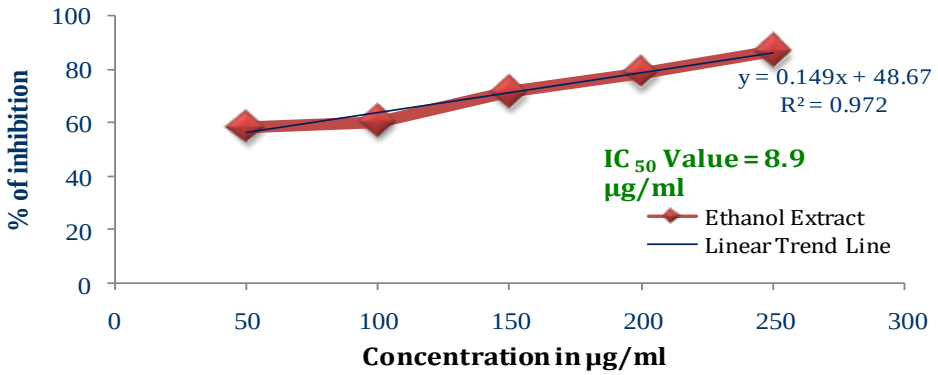

Fig. 5: IC_{50} calculation of *V. articulatum* Burm.f. in ethanol extract

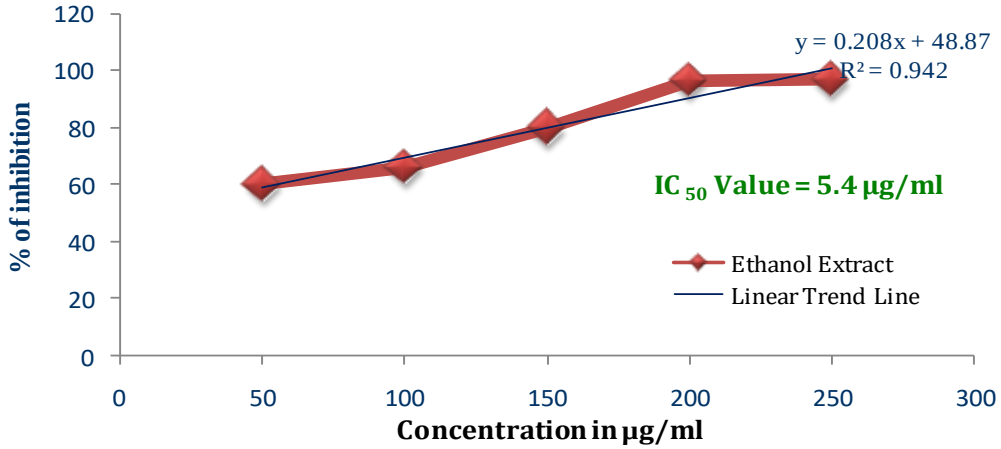

Fig. 6: IC_{50} calculation of Standard (Ascorbic Acid)

Table 4: IC_{50} values of *V. articulatum* Burm.f of different extracts and Ascorbic acid

S. No.	Extracts	IC_{50} values (µg/ml)
1	Acetone extract of *V. articulatum* Burm.f	9.4
2	Ethanolic extract of *V. articulatum* Burm.f	8.9
3	Ascorbic acid	5.4

Using the fitted line, IC_{50} values are calculated, i.e. Y=a*X + b, IC_{50} = (50-b)/a

DISCUSSION

Medicinal plants have played a crucial role in the prevention and the treatment of different ailments since ancient times [25]. In the current research work, I have decided to scientifically explore the uses of *V. articulatum* Burm. f., like to investigate their phytoconstituents and antioxidant activity. In the present work, the phytochemical profile of *V. articulatum* reveals the prominent presence of carbohydrates, cardiac glycosides, proteins, alkaloids, fats, saponin, coumarins, flavonoids, tannins, phenolics, steroids and quinone. The earlier phytochemical reports on *V. articulatum* are still scarce; some are given by Geetha *et al*. [26], Najafi *et al*. [27] and Vadnere *et al*. [28], which also showed the presence of similar phyto-constituents as observed in the present study. Some

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biological activities of these phytoconstituents of *V. articulatum* were also reported [21,26].

The plant has a large number of phyto-constituents such as flavonoid and phenols are reported to exhibit good antioxidant property [27]. In the present work, DPPH radical scavenging assay of *V. articulatum* was studied for antioxidant potential. Since it is a water-soluble free radical scavenger, ascorbic acid has been used as the standard. In addition, in conjuction with the compounds capable of donating reducing equivalents, it regenerates vitamin E in the cell membrane. By donating an electron to the lipid radical to end the lipid peroxidation chain response, ascorbic acid switches to the ascorbate radical [29]. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by decreasing absorbance at 517 nm, which was induced by antioxidants [30]. Lesser, the IC_{50} value more is the antioxidant activity [24].

The findings obtained showed that this plant's ethanolic extract has high antioxidant activity (i.e. IC_{50}= 8.9) compared to that of the acetone extract (i.e. IC_{50} = 9.4) compared to regular ascorbic acid (i.e. IC_{50}= 5.4). Through current methodology, there is no work performed on the antioxidant activity of *V. articulatum* acetone and ethanolic extract. Kannoth *et al*. [21] have reported various assay methods for the single *V. articulatum* methanolic extract and have obtained significant results. So, it is possible to consider the *V. articulatum* plant as an excellent antioxidant.

CONCLUSIONS

The current research work shows that both *V. articulatum* Burm. f. acetone and ethanolic extract, it is a strong source of antioxidant property containing various phytochemicals. The presence of carbohydrates, cardiac glycosides, proteins, alkaloids, fats, saponin, coumarins, flavonoids, tannins, phenolics, steroids and quinone present in these extracts may be responsible for antioxidant activity. From this report, it was concluded that the plant *V. articulatum* Burm.f. has a remarkable antioxidant effect, which may be useful for its novel applications because of its medicinal values.

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CONTRIBUTION OF AUTHORS

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Literature search- Dr. M.R. Kakpure
Writing article- Dr. M.R. Kakpure
Critical review- Dr. M. R. Kakpure
Article editing- Dr. M. R. Kakpure
Final approval- Dr. M. R. Kakpure

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