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In vitro antioxidant investigations on *Baptisia tinctoria* roots

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Abstract
Traditionally, roots of *Baptisia tinctoria* (Wild indigo, Indigo weed; Fabaceae) have been utilized in the Indian frameworks of medication for the treatment of various ailments. The current examination was designed to evaluate antioxidant properties of *B. tinctoria* roots. The antioxidant action was resolved utilizing 1, 1-diphenyl-2-picrylhyrazyl (DPPH) radical strategy. The various extracts of *B. tinctoria* roots were prepared in the increasing order of polarity such as petroleum ether (60-80ºC), chloroform, methanol and water using standard protocols. As polyphenols are the significant class of phytoconstituents present in chosen plant, in this manner it was intended to isolate polyphenolic rich parts from methanol concentrate. The ethyl acetate fraction (IC_{50} = 37.70 µg/ml) of methanol extract of plant roots exhibited maximum antioxidant action followed by methanol extract (IC_{50} = 80.76 µg/ml) and chloroform extract (IC_{50} = 197.36 µg/ml) in comparison to ascorbic acid (IC_{50} = 19.96 µg/ml). On the other side petroleum ether extract (IC_{50} = 475.27 µg/ml) and water extract (IC_{50} = 386.26 µg/ml) did not show any inhibitory impact on stable DPPH radicals. Phytochemical screening of plant roots showed presence of polyphenols. Polyphenols have been considered as common antioxidant agents on the basis of literature data. At last, it is suggested that phenols and flavonoids are answerable for antioxidant action of chosen plant. Further, the column chromatography studies will be planned to isolate these phenols and flavonoids compounds responsible for antioxidant activity.

Keywords: antioxidant, ascorbic acid, *Baptisia tinctoria*, DPPH, flavonoids, phenols

Introduction
Recently, there is an expanded event of different infection like cardiovascular sickness, neurological problems, malignant growth, diabetes and immune system illness because of occurrence of free radicals matters (Kumar *et al* 2014) [4]. These free radicals matters have unpaired electrons and might be delivered from one or the other oxygen or nitrogen known as reactive oxygen species and reactive nitrogen species respectively, prompting lipid peroxidation, direct hindrance of mitochondrial breath and discontinuity of enzymatic chemicals. These reactive species are framed either endogenous or exogenous sources. The endogenous sources incorporate typical breath, macrophages and leucocytes, and exogenous sources incorporate smoking, contaminations, pesticides and organic solvents (Kumar and Dhobi 2017) [3].
The term antioxidant is characterized as the specialist that kills the impact created by free radical. The antioxidants can be grouped into two classes, in particular enzymatic and non-enzymatic. The enzymatic antioxidants agents are created itself in our body where as non-enzymatic antioxidants are gotten from either normal plants or artificial materials which are utilized for the therapy for different infections. Literature reveals that antioxidants from synthetic as well as natural origin can be used to prevent diseases caused by excessive free radical generation. Free radicals include hydroxyl radical, superoxide anion radical and hydrogen peroxide. Highly reactive free radicals which are formed by exogenous chemicals, stress or in the food system are capable of oxidizing biomolecules (Kumar and Dhobi 2017) [3]. Various serious adverse effects like liver harm and mutagenesis are related with the utilization of antioxidants agents got from synthetic sources. The WHO estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicines for their primary health care needs. Hence, scientists are investigating plant based assets to discover more up to date and more secure natural antioxidants (Kumar and Dhobi 2016) [2].
Traditionally, roots of *Baptisia tinctoria* (Wild indigo, Indigo weed; Fabaceae) have been utilized in the Indian frameworks of medication for the treatment of in inability to think, mental confusion, melancholia with stupor, threatened miscarriage from mental depression, and as antiseptic, cathartic and emetic. The various bioactive phytoconstituents such as alkaloids, phenols, flavonoids, coumarins, triterpenes and polysaccharides has been scientifically reported from plant till date (Richa *et al* 2017) [5]. The various scientifically reports of pharmacological activities of plant suggested the treatment of mental and immune system related disorders (Sujata *et al* 2017) [7]. The current examination was designed to evaluate antioxidant properties of *B. tinctoria* roots. The antioxidant profile of plant can be useful for scientist for understanding the job of plant in the counteraction of different infections.

Materials and Methods
Plant Materials
The dried root part of *Baptisia tinctoria* Linn was gathered from K.R. Indo German American company situated in

Kurukshetra, Haryana, India. Dr. Sunita Garg, Head of raw material Herbarium and Museum, NISCAIR, New Delhi. India verified the personality of the plant (Ref. No. NISCAIR/RHMD/Consult/2019/3507-08 dated 14/10/2019.

Chemical and Instrumental Material

The various organic solvents used in present studies such as petroleum ether (60-80ºC), chloroform, methanol and ethyl acetate were purchased from E Merck, Delhi, India. The standard antioxidant drug ascorbic acid was purchased from Hi-media Laboratories Pvt. Ltd. The free radical 1, 1-diphenyl-2-picryl hydrazine (DPPH) was purchased from Sigma-Aldrich, USA. The rotary vacuum evaporator (Perfit, Ambala) was used for recovery of solvents under reduced pressure to concentrate various extracts / fraction. The UV/VIS spectrophotometer (Perfit, Ambala) was used for measuring absorbance of test samples. The distilled water was used in present investigations wherever used. The laboratory grade chemicals were used for extraction technology and analytical grade chemicals were used for DPPH assay.

Preparation of Extracts / Fractions

The roots of plant were washed with normal saline to eliminate earth, dried under daylight and powdered in a processor. Dried and powdered plant materials (500 g) were extracted in the pattern of increasing polarity in a Soxhlet device utilizing solvents such as petroleum ether, chloroform and methanol. The water extract of plant was prepared using reflux method. The solvents were recuperated under vacuum utilizing rotary vacuum evaporator. Dried crude extracts was stored in vacuum desiccator for antioxidant activity (Richa *et al* 2017) [5].

The 25 g of methanol extract of plant was suspended consistently in water, put in a round base flask and divided with ethyl acetate by warming at 50ºC for 30 min alongside persistent mixing. This technique of apportioning with ethyl acetate was repeated for multiple times. Every one of the isolated layers of ethyl acetate were pooled and concentrated under reduced pressure utilizing rotary vacuum evaporator (Sujata *et al* 2017) [7].

***In vitro* Antioxidant Activity**

The stable free radical DPPH was utilized for assurance of free radical inhibitory movement of test samples (Singh *et al* 2016) [6]. The methanolic solution of DPPH (0.1 mM) was prepared immediately before use. The 5 ml of methanolic solution of DPPH (0.1 mM) was added in 50 ml of methanol and kept in dark environment for 30 min at room temperature. The absorbance of solution was determined at 517 nm utilizing UV/VIS spectrophotometer against methanol as blank after 30 min (Chawla *et al* 2016) [1].

Preparation of Standard Solution

The 10 mg standard antioxidant drug named ascorbic acid was weighed and solubilized in 100 ml of methanol to get 100 µg/ml stock solution. Lower dilutions (15, 18, 21, 24, 27 or 30 µg/ml) for ascorbic acid were set up by diluting sequentially with methanol. Equivalent volume of various dilutions of standard were added to methanolic arrangement of DPPH, and kept in dark environment for 30 min at room temperature. The absorbance of solution was determined at 517 nm utilizing UV/VIS spectrophotometer against methanol as blank after 30 min.

Preparation of Test Samples

The various extracts and fraction of plant roots were weighed (250 mg) separately and solubilized in 25 ml of methanol solvent to produce final solution of 10,000 µg/ml concentration. Lower concentrations type dilutions were set up by weakening sequentially with methanol. Equivalent volume of various groupings of test samples were added separately to methanolic arrangement of DPPH, and kept in dark environment for 30 min at room temperature. The absorbance of solution was determined at 517 nm utilizing UV/VIS spectrophotometer against methanol as blank after 30 min.

Percentage free radical inhibitory activity was calculated via using mathematical expression: {[Ac – (As – Ao)] / Ac} × 100. Where Ac = Absorbance of DDPH control, As = Absorbance of test sample / standard + free radical DPPH, Ao = Absorbance of test sample / standard without free radical DPPH interaction. The each observation was estimated in thrice, and free radical inhibitory effect was estimated based on the percentage of DPPH inhibited. IC_{50} values of test samples for antioxidant activity were calculated using standard curve of ascorbic acid.

Results and Discussion

The antioxidant action was resolved utilizing 1, 1-diphenyl-2-picrylhyrazyl (DPPH) radical strategy. This test depends on the estimation of the deficiency of DPPH colour tone at 517 nm after response with test compounds and the response is observed by a spectrophotometer. DPPH is a stable free radical of purple colour and within the presence of antioxidant its shading changes to yellow dependent on the potency of the antioxidant. The absorbance fluctuations in relation to control i.e. 100 % DPPH stable free radical solution is estimated as per cent inhibitory power of free radical. The concentration that induce 50 percent decline in starting initial DPPH free radical concentration is expressed as IC_{50} (Kumar *et al* 2014) [4].

The roots of *B. tinctoria* were defatted by extracting with petroleum ether (60-80ºC) in Soxhlet mechanical assembly. The marcs of plants were then independently extracted with chloroform, methanol and water. The % age yield of various crude extracts such as petroleum ether, chloroform, methanol and water was recorded as 0.65, 2.80, 14.89 and 5.98 % w/w. The various unrefined crude extracts of chosen plant were exposed to standard phytochemical screening strategies to find out different classes of phytoconstituents present in that. The observations of phytochemical screening showed presence of fixed oils in petroleum ether extract; alkaloids, steroids, triterpenoids in chloroform extract; flavonoids, tannins, coumarins in methanol extract and proteins, carbohydrates in water extract. As polyphenols are the significant class of phytoconstituents present in chosen plant, in this manner it was intended to isolate polyphenolic rich parts from methanol concentrate of chosen plants by standard methods and to assess antioxidant action in various concentrates/fraction. The % age yield of ethyl acetate fraction was recorded as 26.80 % w/w with respect to methanol extract.

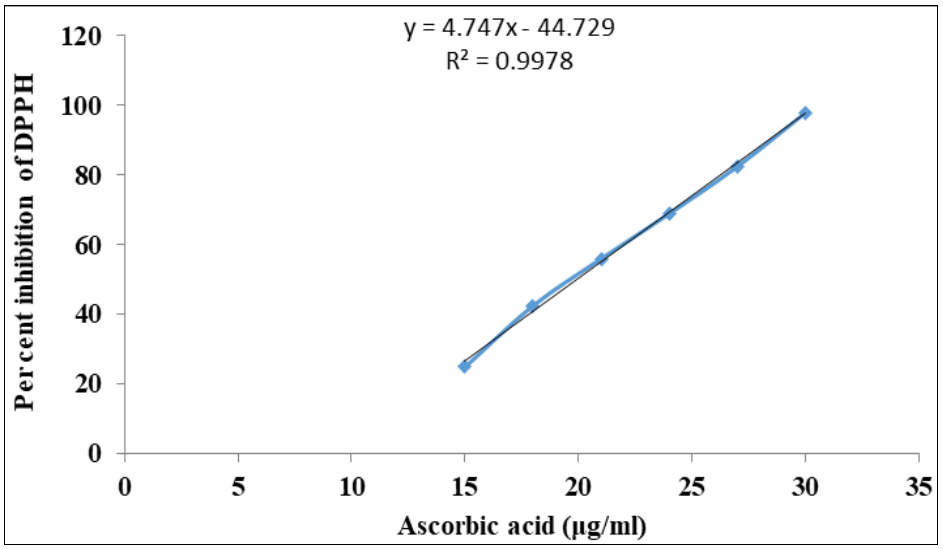
The antioxidant profile of various crude extracts and fraction was expressed in the form of % age inhibitory power of DPPH and IC_{50} values and results are presented in table 1 and figure 1. The antioxidant profile of standard ascorbic acid was established between 15 to 30 µg/ml concentration and coefficient of correlation was recorded as

(r^2) = 0.9978. The ethyl acetate fraction (IC_{50} = 37.70 µg/ml) of methanol extract of plant roots exhibited maximum antioxidant action followed by methanol extract (IC_{50} = 80.76 µg/ml) and chloroform extract (IC_{50} = 197.36 µg/ml) in comparison to ascorbic acid (IC_{50} = 19.96 µg/ml). On the other side petroleum ether extract (IC_{50} = 475.27 µg/ml) and water extract (IC_{50} = 386.26 µg/ml) did not show any inhibitory impact on stable DPPH radicals.

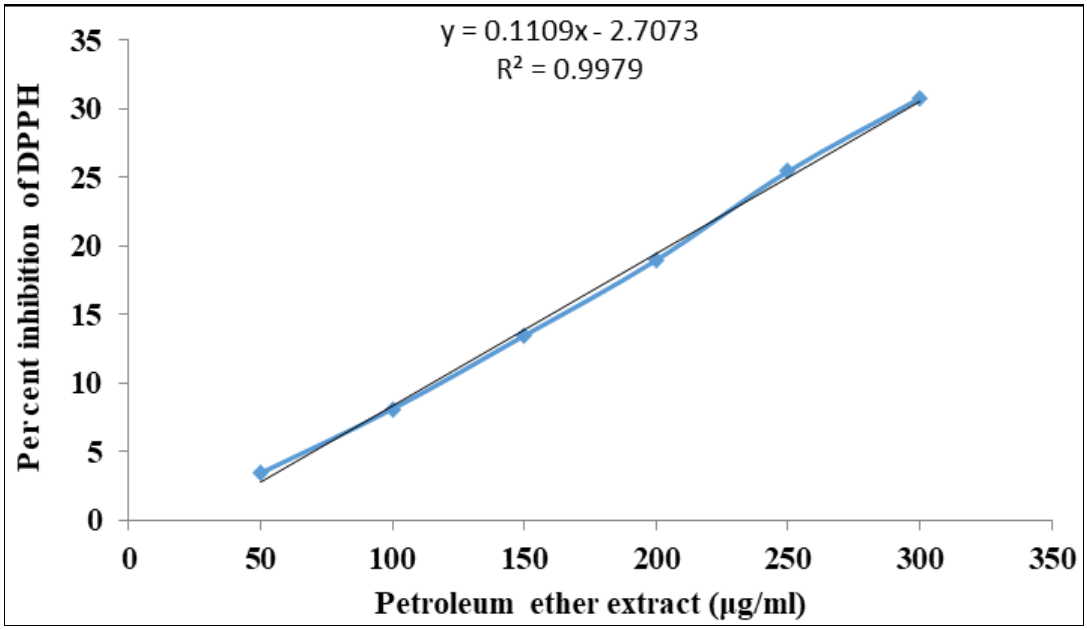
Table 1: Antioxidant profile of various extracts / fraction of plant roots using DPPH assay

Sample	Concentration (µg/ml)	Per cent inhibition	IC50 value
Ascorbic acid	15	24.80 ± 3.01	19.96
	18	42.45 ± 5.78	
	21	55.97 ± 5.28	
	24	68.90 ± 6.12	
	27	82.47 ± 4.78	
	30	97.89 ± 5.70	
Petroleum ether extract	50	3.45 ± 3.47	475.27
	100	8.12 ± 5.45	
	150	13.47 ± 3.88	
	200	18.97 ± 6.89	
	250	25.45 ± 4.78	
	300	30.78 ± 3.77	
Chloroform extract	50	30.45 ± 4.80	197.36
	100	35.89 ± 3.98	
	150	42.74 ± 4.87	
	200	49.80 ± 3.78	
	250	56.98 ± 3.90	
	300	65.40 ± 3.78	
Methanol extract	50	45.80 ± 4.01	80.76
	100	52.47 ± 3.58	
	150	60.45 ± 3.45	
	200	70.94 ± 3.78	
	250	78.90 ± 3.70	
	300	86.97 ± 3.77	
Water extract	50	10.14 ± 3.78	386.26
	100	15.45 ± 3.80	
	150	21.36 ± 6.47	
	200	26.79 ± 5.98	
	250	33.80 ± 6.33	
	300	40.12 ± 3.04	
Ethyl acetate fraction	50	51.14 ± 5.87	37.70
	100	61.45 ± 2.14	
	150	70.80 ± 1.47	
	200	79.89 ± 3.78	
	250	88.97 ± 5.80	
	300	96.64 ± 4.98	

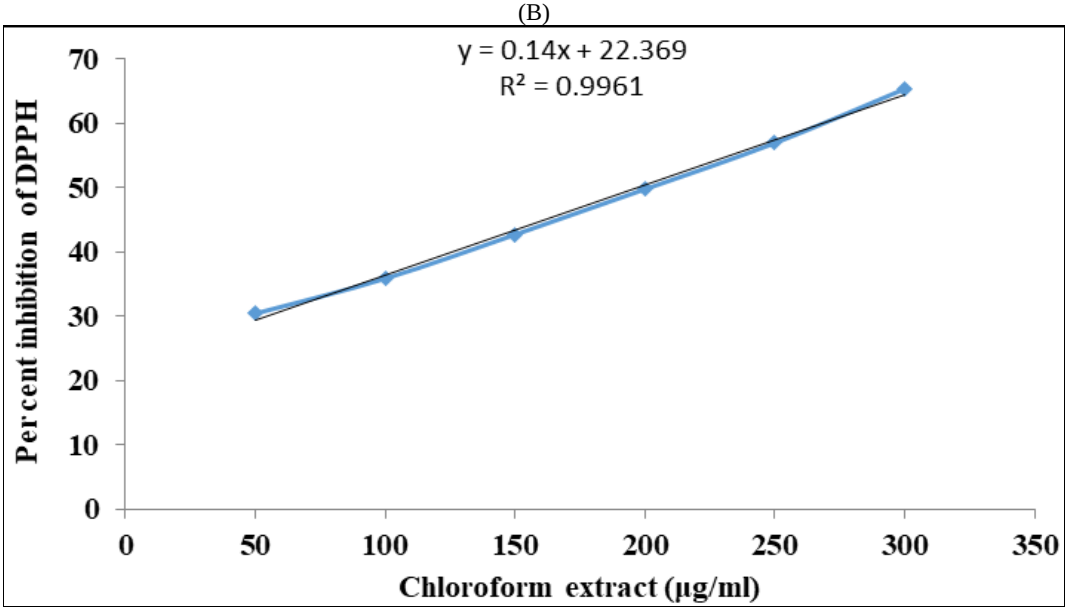
n=3



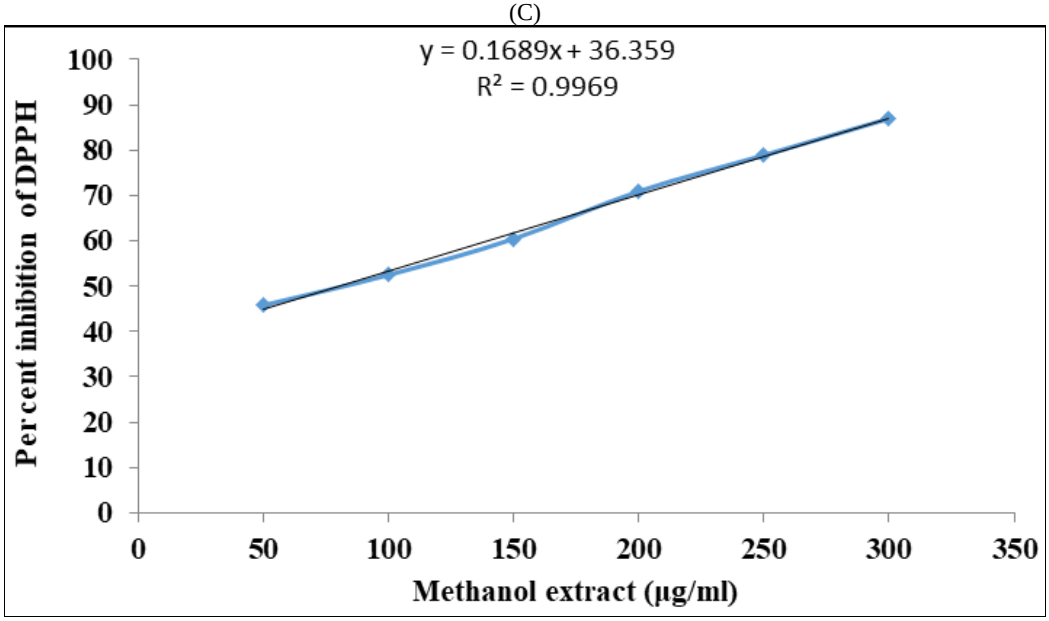
(A)


y = 0.1109x - 2.7073
R² = 0.9979
Percent inhibition of DPPH
Petroleum ether extract (µg/ml)
0
5
10
15
20
25
30
35
0
50
100
150
200
250
300
350

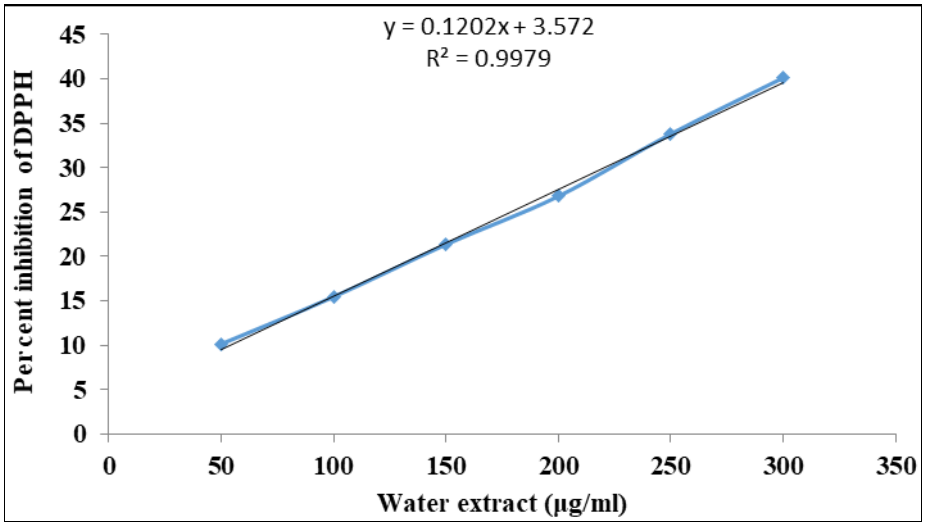
(B)


y = 0.14x + 22.369
R² = 0.9961
Percent inhibition of DPPH
Chloroform extract (µg/ml)
0
10
20
30
40
50
60
70
0
50
100
150
200
250
300
350

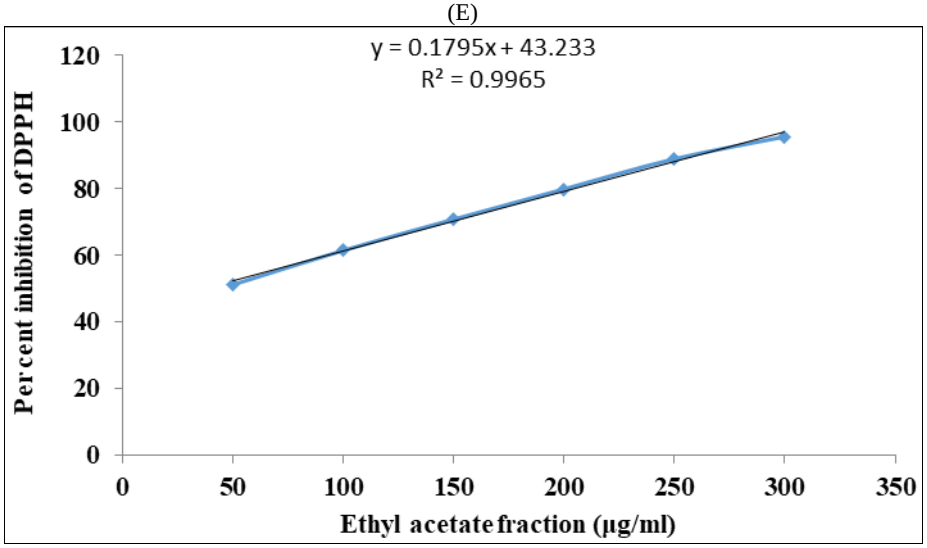
(C)


y = 0.1689x + 36.359
R² = 0.9969
Percent inhibition of DPPH
Methanol extract (µg/ml)
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350

(D)



(E)



(F)

Fig 1: Graphical presentation of antioxidant profile of various extracts / fraction (A-F) of plant roots using DPPH assay

Conclusion

Phytochemical screening of plant roots showed presence of polyphenols. Polyphenols have been considered as common antioxidant agents on the basis of literature data. A close scrutiny of literature suggested that various plants such as *Abies pindrow*, *Abies webbiana*, *Cephalandra indica* and *Calotropis gigantea* exhibited potent antioxidant action due to presence of phenolic and flavonoidal compounds (Kumar *et al* 2014) [4]. At last, it is suggested that phenols and flavonoids are answerable for antioxidant action of chosen plant. Further, the column chromatography studies will be planned to isolate these phenols and flavonoids compounds responsible for antioxidant activity.

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