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Evaluation of antioxidant potential of *Dicoma tomentosa* and *Alhagi maurorum* leaf and stem powder

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ABSTRACT

Dicoma tomentosa and *Alhagi maurorum* are perennial herbaceous plant having multiple medicinal health benefits. The free radicals are generated in the body as metabolic products of several reactions. These free radicals cause multiple harms to the cell, on their cell wall, to the DNA and cause number of diseases. In the present study, the plants were selected and collected from Rajasthan and were shade-dried. The powder was formed of leaves and stem of both the plants. The methanolic extract was prepared for further studies and the enzymatic and non-enzymatic potential of evaluated through their standard protocols. *Dicoma tomentosa* and *Alhagi maurorum* both showed strong antioxidant potential. While comparing both the plants with standard, the stem of *Dicoma tomentosa* was found having comparatively strong antioxidant potential.

Keywords: *Alhagi maurorum*, *Dicoma tomentosa*, antioxidant potential, in-vitro study, comparative evaluation.

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INTRODUCTION:

It is believed that plants have high medicinal values and they are being used for the same from decades. A large number of plants are reported as a remedy for cellular and metabolic disorders. Free radicals are generated inside the body as after product of metabolic reactions. It can also be said that the free radicals are continuously produced by aerobic life. They have generated inside the body as the response of stress. After being generated inside the body these free radicals use to damage the healthy cells by damaging their cell membrane. These free radicals generate due to smoking, excessive exposure to sunlight, because of tobacco, and prolonged exposure of the heavy metals (1).

Effect of a few specific antioxidants is limited maintained inside the, although the antioxidants work to neutralize the side effects generated due to these free radicals. The ratio of oxidants and antioxidant must be maintained inside the body, any kind of alteration in this ration can make the free radicals get deposited inside the body and results in oxidative stress. This oxidative stress can cause major tissue damage which results in cancer (2). Role of oxidative stress can be defined such that it plays a deciding role in carcinogenesis and also a very crucial role in the last stage of cancer for its progression and prevention. The oxidative stress can be defined as diligence imbalance in the antioxidant/pro-oxidant level, which results in unidirectional cell damage. The antioxidants scavenge the generated free radicals and inhibit the damage caused due to oxidative stress (3, 4). In the present study, we evaluated the antioxidant potential of *Alhagi maurorum* and *Dicoma tomentosa* steam and leaf powder. *Alhagi maurorum* is considered as a renowned folk medicine used against liver disorders, jaundice, arthritis, rheumatic pains, respiratory problems, wounds, urinary infections, dysentery, hemorrhoids, antimicrobial and also aphrodisiac (5,6). Whereas *Dicoma tomentosa* is a renowned medicinal herb known as vajradanti, belongs to family *Compositae* having various medicinal benefits along with antimicrobial, levoprotective, renoprotective, cardioprotective activities (7).

MATERIALS AND METHODS:

The plant material was collected from (Udaipurwati, Jhunjhunu and Mehlan, Jaipur) Rajasthan and was authenticated in Department of Botany, the University of Rajasthan during September and October 2017. The crude extract was obtained by extraction. The 10 gm of dried powder of both the plants was macerated in 95% of methanol and it was kept in rotatory soxhlet apparatus for

24 hours. Forwarding on the next step the extract was filtered and dried. It was stored in an airtight bottle at 40 ^{0}C. The dried extract was further dissolved in the solution of dimethyl sulfoxide for preparation of final concentration and was kept in 4 ^{o}C till being used. The extract was stored at 40 ^{o}C in an airtight bottle.

DPPH radical scavenging activity

DPPH assay was measured by the method proposed by Mensor. For estimation of antioxidant potential by Mensor 20µl of concentration of methanolic extract of dried powder of both the plants were taken (8). The extract of both the plants was added in methanolic solution 0.5ml of DPPH. The mixture was allowed to set at RT (room temperature) for 30 minutes. Methanol was taken as blank; DPPH in methanol was taken as control (positive control). The spectrometric analysis was done and after incubation, a purple color was obtained, OD was taken at 518nm.

FRAP radical scavenging activity

FRAP was carried out by the method reported by Benzie *et al.,* 1996. The stock solution was prepared using 300mM of acetate buffer with pH 3.6, TPTZ solution (2, 4, 6- tripyridyl-s-triazine) in HCL 40 mM and FeCl3 20 mM solution. From stock solution, working solution was prepared fresh by 2,5 ml TPTZ in 25 ml of acetate buffer and 2.5ml of FeCl3.6H2O solution. The working solution was kept at (RT) 37°C. Different solutions of plant extracts of both the plants were taken and mixed in 2850 ml of FRAP solution; the mixture was kept in dark for 30 minutes for incubation. The color was observed and OD was measured at 593 nm. The results were expressed on the basis of antioxidant concentration with the ability to reduce ferric ion (9).

Nitric oxide radical scavenging activity

NO- inhibition was studied by the method of Makchuchit *et al.,* 2010. The reaction was started by addition sodium nitroprusside 2.0 ml of 100 mM, 0.5 ml of herbal extracts and 0.5ml of phosphate buffer with pH 7.4. In this mixture 0.5ml of Griess reagent was mixed and allowed to incubate for 30 minutes at RT. Control was having sodium nitroprusside, herbal extract, and Griess reagent. Samples were prepared in triplicate and absorbance was taken at 546 nm. The absorbance was taken of chromophore formed as result of diazotization of sulphanilamide and naphthyl ethylenediamine coupling (10).

Hydrogen peroxide radical scavenging activity

Estimation of hydrogen peroxide was done by Ruch *et al.,*1998. The 06 ml of H_2 O_2 solution was taken and herbal methanolic extracts were mixed in it. 40mm of H_2 O_2 was prepared by the help of phosphate buffer, the pH was set neutral 7.4 and the solution was 0.1 M. Total volume of the mixture was made up to 3 ml and the absorbance was taken at 230 nm. To perform the test phosphate buffer with H_2O_2 was taken as blank (11).

Superoxide radical scavenging activity

SOD was measured by the method of Suprava *et al.,* 2013. SOD ion was generated in a sample containing 0.2 ml of the plant extracts, 0.2 ml of EDTA having concentration 0.1 M in 1.5 mg of NaCN with 0.1ml of NBT (nitro blue tetrazolium), phosphate buffer 2.64 ml, riboflavin 0.12mM. Control taken for the study was DMSO. The tubes were vortexed and OD was measured at 560 nm. The next step was exploring the tubes to fluorescent light for 30 minutes after 30 minutes of exploration absorbance was measured at 560nm. The difference before and after illumination was measured, this difference indicates the scavenging activity of SOD (12).

Lipid peroxidation activity:

Lipid peroxidase was measured by Dudonne *et al.,* 2009 in the herbal extracts of both the plants. 0.1 ml of herbal extracts, with deionized water and homogenate of egg in 0.2 M of PBS was taken. FeSO4 was added into the mixture and left for 37 ^{o}C for incubation. In this incubation time, lipid per-oxidation was allowed to induce. The tubes were further filled with a mixture of 20% acetic acid, 20 % TCA and 0.8% TBA and the mixture was heated for 60 minutes in boiling water bath. The mixture was further allowed to cooling, after cooling 5ml butane-1-ol was added and the tubes were centrifuged for 3000rpm, the supernatant was removed safely in a separate test tube and the OD of the supernatant was measured spectrophotometrically at 532 nm. Control was taken without herbal extracts. IC 50 value and inhibition percentage were calculated through the graph (13).

Catalase:

The antioxidant potential through catalase was measured by Aebi *et al.,* 1984. 50 µl of lysate of herbal extracts was taken and mixed with 2 ml of phosphate buffer and 1 ml H_2 O_2. The activity of catalase was measured spectrophotometrically and OD was measured at 240nm for the duration of 1 minute. For determination of the activity of catalase molar extinction capacity was measured as unit/mg of protein (14).

RESULTS

Table 1. In-vitro non-enzymatic antioxidant activity of methanolic extract prepared from dry powder of leaf and stem of *Alhagi maurorum* and *Dicoma tomentosa.*

S. No	Assay	OD (nm)	*Alhagi maurorum*		*Dicoma tomentosa*	
			Leaf	Stem	Leaf	Stem
1.	DPPH	518	0.63	0.73	0.81	0.85
2.	FRAP	593	0.31	0.66	0.76	0.49
3.	NO	546	0.84	0.81	0.65	0.66
4.	LPO	532	0.91	0.90	0.96	0.98

Note: - The values were obtained by independent determination in triplicate where n= 3, one way ANOVA was applied for statical calculation the mean was taken and the values showed significant difference where $p<0.005$.

Table 2. In-vitro enzymatic antioxidant activity of methanolic extract prepared from dry powder of leaf and stem of *Alhagi maurorum* and *Dicoma tomentosa*.

S. No	Assay	OD	*Alhagi maurorum*		*Dicoma tomentosa*	
			Leaf	Stem	Leaf	Stem
1.	CAT	240	1.08	1.66	1.72	2.74
2.	POD	430	0.86	0.28	1.92	2.74
3.	SOD	543	0.70	0.23	0.85	0.86

The values were obtained by independent determination in triplicate where n= 3, one way ANOVA was applied for stastical calculation the mean was taken and the values showed significant difference where p<0.005.

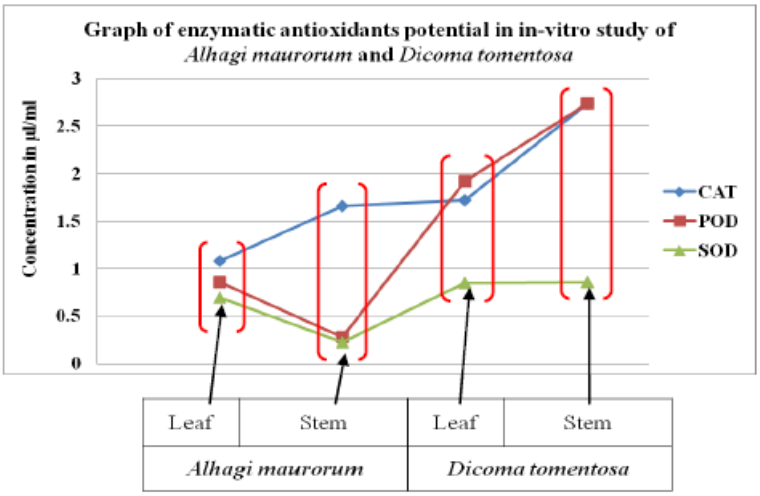
Note: - CAT – Catalase; POD – Peroxidase; SOD – Superoxide dismutase.

Table 3. Free radical scavenging activity of methanolic extract of dried leaf and stem parts of *Alhagi maurorum* and *Dicoma tomentosa*.

	Assay	Con mg/ml	*Alhagi maurorum*				*Dicoma tomentosa*				Standard	
			Leaf		Stem		Leaf		Stem			
			Percentage	Mean SD	Percentage	Mean SD	Percentage	Mean SD	Percentage	Mean SD	Percentage	Mean SD
1.	DPPH	50	73.3	0.61±0.001	88.1	0.71±0.001	81	0.81±0.001	85	0.85±0.001	83%	0.289±0.001
2.	FRAP	50	69	0.31±0.001	59	0.66±0.001	83	0.71±0.001	80	0.43±0.001	76%	0.593±0.003
3.	NO	50	78	0.81±0.001	75	0.80±0.001	80	0.60±0.001	81	0.67±0.001	80%	0.453±0.001
4.	LPO	50	65	0.90±0.001	63	0.89±0.001	75	0.91±0.001	71	0.94±0.001	76%	0.221±0.001
5.	CAT	50	53	1.06±0.001	49	1.04±0.001	48	1.69±0.001	78	1.70±0.001	80%	2.450±0.001
6.	POD	50	56	0.84±0.001	59	0.22±0.001	60	1.94±0.001	66	2.65±0.001	56%	0.360±0.001
7.	SOD	50	68	0.71±0.001	45	0.20±0.001	76	0.81±0.001	79	0.84±0.001	80%	0.293±0.001

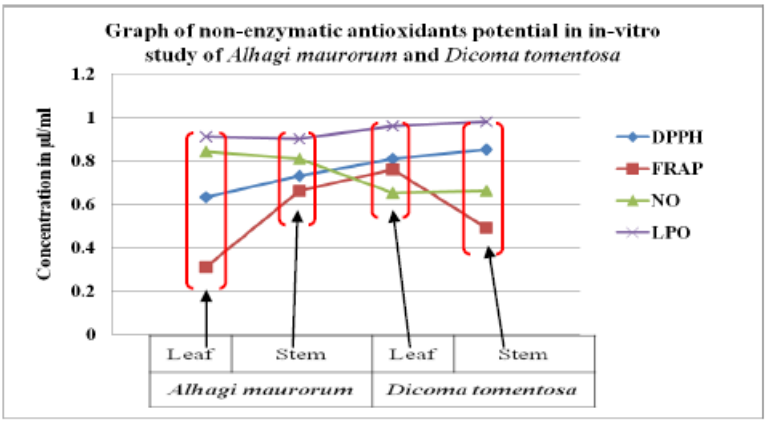
The values were obtained by independent determination in triplicate where n = 3, one way ANOVA was applied for stastical calculation the mean was taken and the values showed significant difference where p<0.005.

Graph 1.



Graph 1. Showing free radical scavenging activity of *Alhagi maurorum* and *Dicoma tomentosa* dried stem and leaves powder, both the plants potent antioxidant potential where *Dicoma tomentosa* stem powder methanolic extract showed higher concentration of Catalase and Peroxidase assay and moderate concentration of SOD (super oxide dismutase).

Graph 2.



Graph 2. Showing free radical scavenging activity of *Alhagi maurorum* and *Dicoma tomentosa* dried stem and leaves powder, both the plants potent antioxidant potential where *Dicoma tomentosa* stem and leaf powder methanolic extract showed higher concentration of DPPH, FRAP and LPO (lipid per oxidation). Whereas moderate increament was seen in values of NO (Nitric oxide).

While compiling different methods of evaluation of free radical scavenging activity different assay where applied, among them four non-enzymatic and three enzymatic parameters were studied in dried powder extracts of *Alhagi maurorum* and *Dicoma tomentosa* leaf and stem tissues. Both the plants showed potent antioxidant activity. *Dicoma tomentosa* stem and leaf dried powdered methanolic extract showed better free radical scavenging activity in comparison to *Alhagi maurorum* dried powder extract (methanolic extract). Higher values of catalase and peroxidase were seen in the stem of *Dicoma tomentosa* and a moderate increment of SOD value was seen in leaves and stem of *Dicoma tomentosa* plant (table 2, graph 1). While evaluation of non-enzymatic free radical scavenging assay higher values of DPPH, FRAP and LPO were obtained and moderate increment of NO was seen. Both the stem and leaves of *Dicoma tomentosa* showed similar values (table 1, graph2).

While evaluating the percentage wise free radical scavenging capacity of both the plants enzymatically and non enzymatically, in comparison to a standard, *Dicoma tomentosa* showed better results in comparison to *Alhagi maurorum* (table 3). Better comparative results were obtained of DPPH was seen in the stem of *Alhagi maurorum* and leaves and stems of *Dicoma* tomentosa. While comparing the free radical scavenging percentage of FRAP assay *Dicoma tomentosa* leaves and stem showed better inhibition. On evaluating the LPO and NO the dried powdered methanoic extracts of *Dicoma tomentosa* showed a higher percentage of free radical scavenging. A higher percentage of inhibition of generated free radicals was seen in *Dicoma tomentosa* stem and leaves in comparison of standard and another studied plant *Alhagi maurorum* catalase, superoxide dismutase, and peroxidase showed better percentage. Peroxidase value was seen comparatively lower in the stem of *Dicoma tomentosa* (table 3).

DISCUSSION:

Antioxidants play a vital role in scavenging freer radicals generated inside the body by synthetic antioxidants butylated hydroxyanisole and butylated hydroxytoluene (15). These antioxidants provide immerse support for defense against generated free radicals mediated diseases (16). Folk medicines have a vital antioxidant potential and work as potent antioxidant agents. Different enzymatic and non-enzymatic antioxidants were analyzed in stem and leaf dried tissues of Alhagi *maurorum* and *Dicoma tomentosa*. Hydrogen peroxide gets generated when superoxide radical get dismutated in presence of enzyme SOD. As a result of this reaction, H_2O_2 get generated, which is more toxic for cell. Peroxidase and catalase also showed increased antioxidant potential due to an increase in SOD activity (17). All medicinal plant has few molecules which work to exhibit free radical scavenging activity; these molecules are flavonoids, alkaloids, tocopherol, lycopene, and phenolics. The antioxidants considered as non- enzymatic are found essential for redox buffering (18). In antioxidant analysis through FRAP is based on the principal of ferric-tripyridyltriazine reduced to ferrous ion complex as a resultant blue color is formed, this blue color is directly associated with antioxidant inductance of the sample. The herbal extract was found capable to donate electrons to the tissues and cell to stabilize the nonreactivity of the radicals. As the absorbance increases the reduction capacity of the generated free radicals also increases. Increased capacity of free radical reduction also inhibits catalase and LPO activities.

In a few diseases on their progression, NO is produced, which results in nitrate and nitrite in the presence of O_2. The methanolic extracts of both the plants exhibited proficient antioxidant activity and the generate oxygen reacts with NO and inhibit produced nitrite. Similarly on analyzing the role of peroxidase H_2O_2 was found most reactive and harmful for cells internal immunity. Here SOD serves as a precursor for singlet hydroxyl radical and oxygen. The rate of inhibition of these radicals is inhibited by NBT inhibition by SOD. SOD is found in both anaerobic and aerobic organisms (19). The methanolic extract of dried leaves and stem of Alhagi maurorum and *Dicoma tomentosa* reported having higher activity of ferrous ion. The high content of phenolic compounds was found effective as antioxidant and quench free oxygen; it inhibits oxidation of lipid and different biological molecules (20).

CONCLUSION:

On concluding the study, both the studied medicinal plants showed potent antioxidant potential, where *Dicoma tomentosa* was found more potent antioxidant herb as compared to Alhagi maurorum. The dried stem powder of *Dicoma tomentosa* showed more free radical scavenging activity. The results reveal medicinal importance of both the plants due to their phytoconstituents and both the plants were proved to be a good source of antioxidants. Only a little

data is available on *Dicoma tomentosa*. Purpose of the present study is to evaluate a comparative antioxidant potential of both the studied plants.

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