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Studies on the Antioxidant Properties of Various extracts of *Hippophae rhamnoide*

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Abstract*—Sea Buckthorn (Hippophae rhamnoides) a spiny shrub native to Ladakh Region of Jammu and Kashmir, have been found to posses so many medicinal properties from times immoral. From this point of view the antioxidant property of the plant fruit extracts have been analysed by DPPH method. Various plant extracts viz, fruit, leaf and root have been analysed for the antioxidant power determination in which fruit extracts showed highest free radical scavenging activity followed by leaf and root extracts. Among the solvents which have been used, more polar solvents showed highest antioxidant activity than the less polar solvent extracts. The IC_{50} value of various plant extracts as determined have been found to be 40 for DCM extract of fruit, 38 for Methanolic extract of fruit and 30 for the water extract of fruit. Similarly the leaf extracts posses IC_{50} value as 51, 47 and 37 respectively for DCM, Methanol and Water extracts. The IC_{50} values of various root extracts have been found to be 53, 50 and 48 respectively for DCM, Methanol and Water.*

***Keywords*— antioxidants, DCM extract, methanol extracts, water extract, Hippophae rhamnoides, DPPH (2, 2-diphenyl-1-picrylhydrazyl).**

I. INTRODUCTION

Sea Buckthorn (SBT) is a deciduous, branched, spiny shrub belonging to genus *Hippophae* and family Elaeagnaceae which usually forms shrub 3 to 15 feet height although some SBT (*Hippophae rhamnoides*) in China have reached 18 m (59 feet), and others grow no higher than 50 cm (20 inches). Before 12 century BC, the ancient Greeks surprised to find some sick horses loose to die a natural death became strong and energetic again. They found the source of this magic was traced to sea buckthorn and named the shrub *H. rhamnoides* L., meaning trees that make horse shine. The plant are considered to be rich source of a large number of bioactive substances like flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glycoside compounds), carotenoids (β and δ-carotene, lycopene, Zeaxanthin), few essential amino acids, sitosterol, triterpene, fatty acids, tannin acid, 5-hydroxytryptamine, umbelliferone, antioxidant vitamins and minerals.1,2,3. SBT has been called a wonder plant in many Asian countries, including China, India, and Pakistan. SBT is a particular and valuable plant species, currently domesticated in various parts of the world reflecting interest in its long-identified multiple uses. In India species of Hippophae grow in five states; 3 in the North-West (Lahaul-Spiti districts of Himachal Pradesh, Uttaranchal and river belts of Indus, Nubra, Shyok, Zanskar etc. of ladakh) and 2 in the North-East (Sikkim and Arunachal Pradesh) Himalaya4. The classification of genus Hippophae is still unclear although it has been classified into seven major species; *H. tibetana, H. salicifolia, H. rhamnoides, H. neurocarpa, H. litangensis, H. gyantsensis and H. goniocarpa*. In India, *H. rhamnoides, H. salicifolia* and *H. tibetana* have been described. Of which *H. rhamnoides L. ssp. Turkestanica* are the major one 5. The leaves of the plant are used in Middle Asia for GI and skin disorders, topically applied to treat rheumatoid arthritis. The present review is an attempt to assess the research activities of SBT related to health benefits.

Anand Vijayan Menon, Vijayalakshmi S, Ranjitha J (1) investigated the preliminary phytochemical screening of the leaves of *Hippophae rhamnoides L* belonging to family *Elaeagnaceae*. All the extracts were subjected to qualitative phytochemical screening and it showed the presence of active constituents such as alkaloid, flavonoids, phenol and tri-terpenoids. Alam Zeb (2) showed that Sea buckthorn juice is one of the imperative product obtained from the sea buckthorn berries, is now commercially very important. The juice provides a nutritious beverage, high in suspended solids, and very high in vitamins especially in vitamin C and carotenoids. SAADIA CHAMAN, NAWAZISH-I-HUSAIN SYED (3) showed Sea buckthorn berries are therapeutically used as folk medicine for a variety of diseases however; the scientific evidence is hardly available to support their role. SIVARAJ ANBARASU, MANIKKAM RADHAKRISHNAN (4) listed that Sea Buckthorn (SBT) parts are used traditionally for several

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ailments. Medicinally, it has been proven to possess various pharmacological activities such as antioxidant, antimicrobial, antifungal, metabolic disorders, immune stimulatory activity, hepato protectant and anticancer activity. Manjari Bhartee, B. C. Basistha and Sushen Pradhan *Hippophae* L. (5) showed that this is a multipurpose wonder plant found in the Himalayan region which is beneficial both ecologically and economically.

II. EXPERIMENTAL

The *Hippophae rhamnoides* plant was collected from the Ladhkah Region of Jammu and Kashmir. The plant was shade dried and powdered in to mixture.

Extraction

50 gms of the plant root, stem and leaf powder were weighed separately and accurately and then extracted in a Soxhlet Apparatus using thimble in order to get the best extract. Various solvents were used depending upon their polarity index with increasing polarity (DCM, Methanol and Water).

Extraction A: The sample was extracted with a particular solvent (DCM) in a Soxhlet apparatus for a required period. After the Extraction with Petroleum ether, the extract solution was subjected to filtration to remove the residue from extract. The filtrate was then collected and evaporated to remove the volatile solvent to its $1/4^{th}$ volume on water bath at a suitable temperature. The whole filtrate was then made in solid form (powdered) after being kept in an oven at 40-60^{0}C. The residue was collected, and subjected to further extraction process.

Extraction B: The residue was then extracted with Methanol in a same manner as mentioned above, in extraction A.

Extraction C: The residue from extract B was subjected to Water extraction by decoction technique. In this technique the extract was dissolved in 500 ml of water. The whole solution was heated over water bath to remove all the water from the extract. Finally additional 500 ml of water was added to the extract, the extracted solution was finally evaporated to remove nearly 250 ml of water. The obtained solution was subjected to filtration and then the filtrate was evaporated to remove nearly 1/4th of its volume. Finally the extract was dried in an oven at a temperature range 30-50^{0}C.

ANTIOXIDANT ACTIVITY

DPPH Method

DPPH Scavenging activity was measured by the Spectrophotometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol. Decrease in the absorbance in presence of sample extract at different concentration (10-125 µg/ml) was noted after 15 min. IC_{50} was calculated from % inhibition.

Protocol for DPPH Free Radical Scavenging Activity

Preparation of stock solution of the sample:-

100 mg of extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution.

(1) Dilution of test solution: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml solution of test were prepared from stock solution.

(2) Preparation of DPPH solution: 15 mg of DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminum foil to protect from light.

(3) Estimation of DPPH scavenging activity: 75 µl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 100 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration. Final decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm. Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

% Reduction = Control absorbance – Test absorbance/ Control absorbance x 100

Calculation of **IC_{50}** value using graphical method.

Observations

Antioxidant Power of *Hippophae rhamnoides*

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Table and Graph 1: DPPH Free Radical Scavenging Activity of Ascorbic Acid

Absorbance of the sample at 517nm

Absorbance of Control = 0.490

S .No.	Conc. (µg/ml)	Abs. of Ascorbic acid	% Reduction	IC_{50} Value (µg/ml)
1.	10	0.292	40.63	26
2.	20	0.269	45.90	
3.	30	0.244	50.60	
4.	40	0.226	54.45	
5.	50	0.202	59.10	
6.	60	0.181	63.33	
7.	70	0.162	67.21	
8.	80	0.141	71.45	
9.	90	0.122	75.30	
10.	100	0.088	82.16	

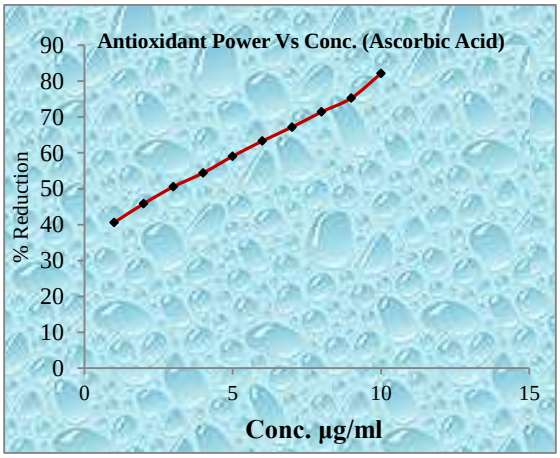

Table and Graph 2: Antioxidant Activity of Fruit DCM Extract of Hippophae rhamnoides

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.280	37.71	40
2.	20	0.270	41.86	
3.	30	0.250	43.31	
4.	40	0.230	50.45	
5.	50	0.210	55.13	
6.	60	0.180	60.85	
7.	70	0.149	68.11	
8.	80	0.130	71.22	
9.	90	0.111	79.40	

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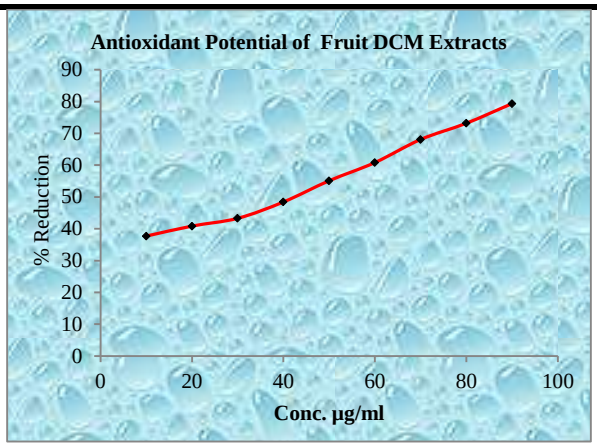

Table and Graph 3: Antioxidant Activity of Fruit Methanol Extract of Hippophae rhamnoides

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.272	36.73	38
2.	20	0.259	41.83	
3.	30	0.234	45.31	
4.	40	0.216	51.42	
5.	50	0.204	57.14	
6.	60	0.175	62.85	
7.	70	0.158	69.18	
8.	80	0.135	73.26	
9.	90	0.121	79.38	
10.	100	0.079	81.63	

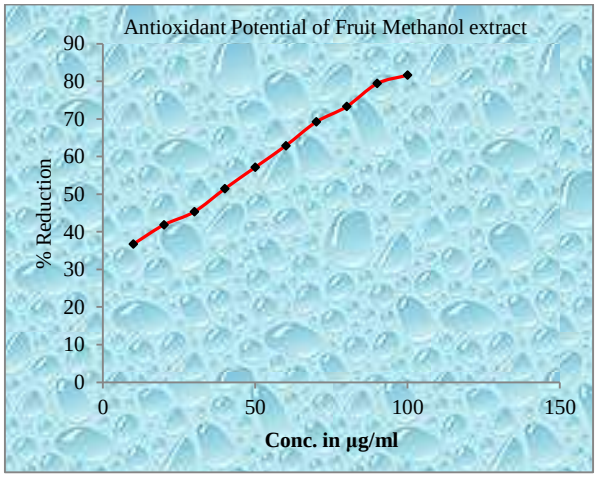

Table and Graph 4: Antioxidant Activity of Fruit Water Extract of Hippophae rhamnoides

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.288	43.82	30
2.	20	0.261	46.93	

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3.	**30**	**0.241**	**49.38**	
4.	**40**	**0.230**	**52.65**	
5.	**50**	**0.211**	**57.14**	
6.	**60**	**0.181**	**61.22**	
7.	**70**	**0.162**	**66.32**	
8.	**80**	**0.141**	**71.42**	
9.	**90**	**0.117**	**73.26**	
10.	**100**	**0.072**	**77.55**	

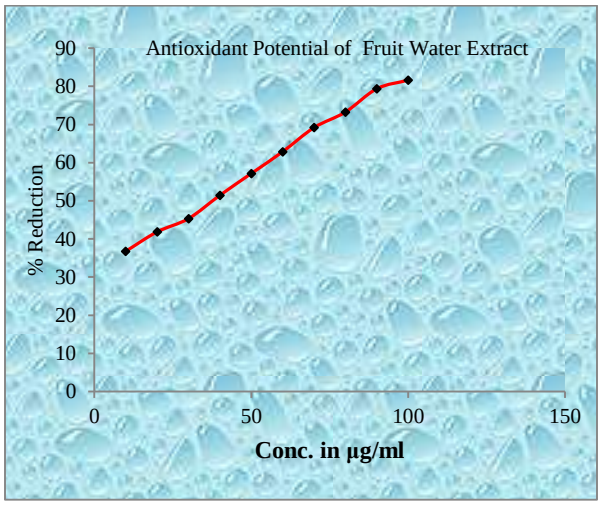

Table and Graph 5: Antioxidant Activity of Leaf DCM Extract of Hippophae rhamnoides

S. No	**Conc. (µg/ml)**	**Absorb**	**% Reduction**	**IC_{50} Value**
1.	**10**	**0.277**	**43.46**	
2.	**20**	**0.270**	**44.89**	
3.	**30**	**0.265**	**45.91**	
4.	**40**	**0.254**	**48.16**	
5.	**50**	**0.249**	**49.18**	
6.	**60**	**0.241**	**50.81**	**51**
7.	**70**	**0.217**	**55.71**	
8.	**80**	**0.190**	**61.22**	
9.	**90**	**0.177**	**63.87**	
10.	**100**	**0.164**	**66.53**	

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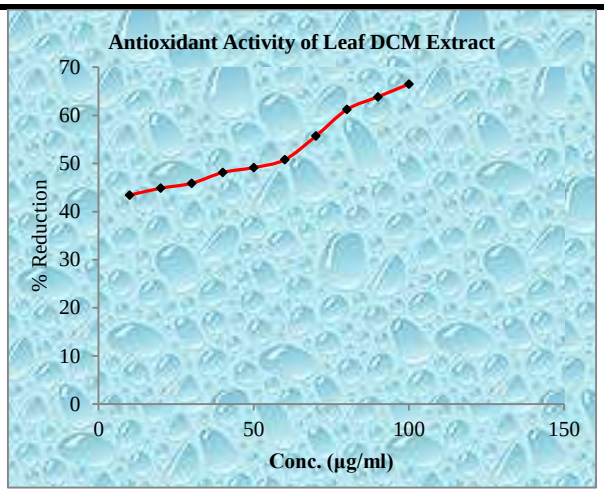

Table and Graph 6: Antioxidant Activity of Leaf Methanol Extract of Hippophae rhamnoides

S. No	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.370	24.48	47
2.	20	0.353	27.95	
3.	30	0.321	34.48	
4.	40	0.286	41.63	
5.	50	0.255	48.97	
6.	60	0.216	55.91	
7.	70	0.192	60.81	
8.	80	0.165	66.32	
9.	90	0.128	73.87	
10.	100	0.102	79.18	

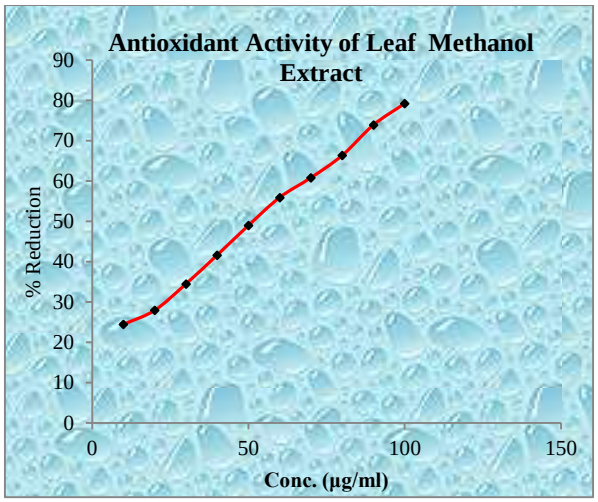

Table 7: Antioxidant Power of Leaf Water extract of Hippophae rhamnoides

S. No	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.272	44.48	37

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2.	**20**	**0.260**	**46.93**	
3.	**30**	**0.258**	**47.34**	
4.	**40**	**0.248**	**49.38**	
5.	**50**	**0.210**	**57.14**	
6.	**60**	**0.175**	**64.28**	
7.	**70**	**0.160**	**67.34**	
8.	**80**	**0.140**	**71.42**	
9.	**90**	**0.131**	**73.26**	
10.	**100**	**0.121**	**75.30**	

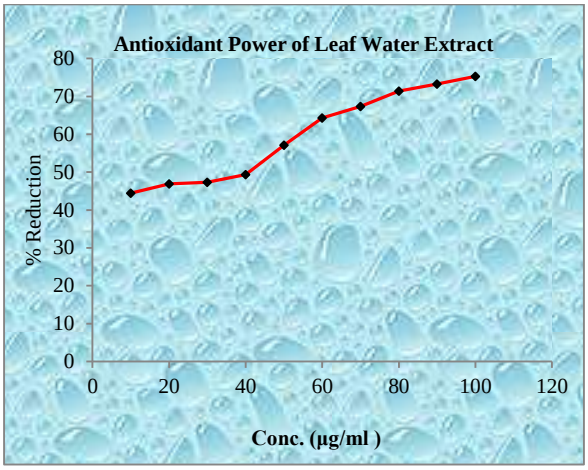

Table 8: Antioxidant Power of Root DCM extract of Hippophae rhamnoides

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	**10**	**0.282**	**42.44**	**53**
2.	**20**	**0.272**	**44.48**	
3.	**30**	**0.264**	**46.12**	
4.	**40**	**0.251**	**48.77**	
5.	**50**	**0.210**	**57.14**	
6.	**60**	**0.191**	**61.02**	
7.	**70**	**0.170**	**65.30**	
8.	**80**	**0.150**	**69.38**	
9.	**90**	**0.130**	**73.46**	
10.	**100**	**0.123**	**74.89**	

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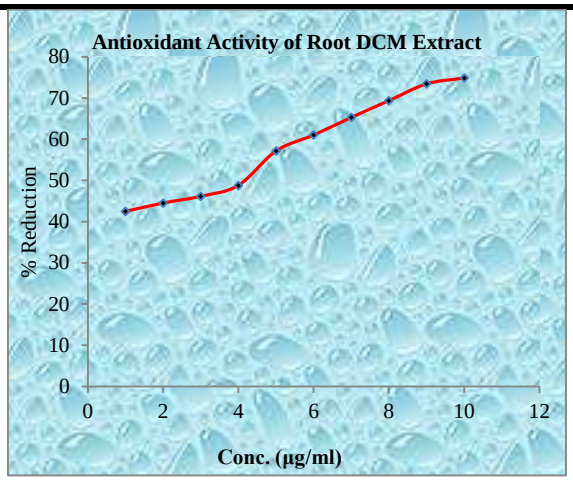

Table and Graph 9: Antioxidant Activity of Root Methanol Extract of Hippophae rhamnoides

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.290	40.81	50
2.	20	0.281	42.65	
3.	30	0.273	44.28	
4.	40	0.260	46.96	
5.	50	0.230	53.06	
6.	60	0.215	56.12	
7.	70	0.197	59.79	
8.	80	0.175	64.28	
9.	90	0.169	65.51	
10.	100	0.154	68.57	

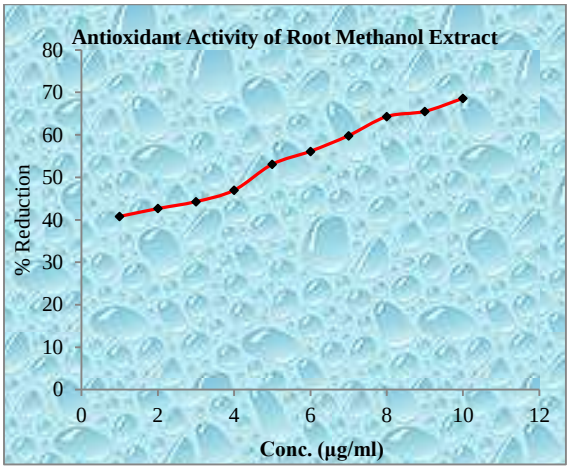

Table 10: Antioxidant Activity of Root Water Extract of Hippophae rhamnoides

S. No.	Conc. (µg/ml)	Absorb	% Reduction	IC_{50} Value
1.	10	0.380	22.44	48
2.	20	0.351	28.36	

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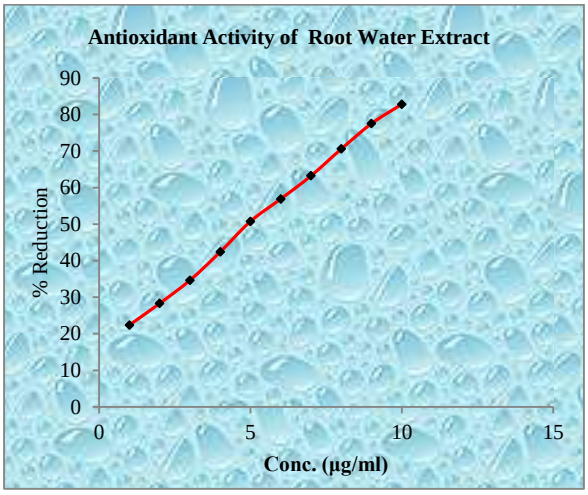
3.	**30**	**0.320**	**34.69**	
4.	**40**	**0.282**	**42.44**	
5.	**50**	**0.241**	**50.81**	
6.	**60**	**0.211**	**56.93**	
7.	**70**	**0.180**	**63.26**	
8.	**80**	**0.144**	**70.61**	
9.	**90**	**0.110**	**77.55**	
10.	**100**	**0.084**	**82.85**	



III. DISCUSSION

The kingdom Plantae is very interesting, because it furnishes all the types of metabolites which are very useful for the continuity of life, and for good health in addition helps in making our environment clean.

The human biology is very complex system in nature. Its functional biology is contingent upon the simultaneous production and elimination of free radicals produced during the various biomechanisms operating in the body system, excessing the free radical concentration to the threshold level. The threshold crossing levels of free radicals concentration in th body invites the various types of diseases in the body like diabetes, Parkinsons disease, cancer to name a few. The nature has provided the different types of bio-organics available inherently in the forest wealth and vegetable to combat the overproduction of free radicals in the body system.

The study (free radicals, scavenging) activity of the plant were taken into consideration. As per the results, the plant showed a good response towards free radicals scavenging activity.

The antioxidant power of the Fruit, Leaf and Root extracts of ***Hippophae rhamnoides*** had been revealed by DPPH method in which it had been found that all plant extracts show that the % reduction increases with the increase in the concentration of the stem extracts. The fruit extracts **(Figs 2-4)** show marginally higher antioxidant potential than the other leaf and root extracts**.** The antioxidant powers of all the plant extracts have been found to be less than the Ascorbic acid. The three plant part extracts follow the order as **(Fruit › Leaf › Root).** All the plant extracts have been found to be concentration dependent as the % reduction increases correspondingly with the increase in the concentration of extracts. A straight line had been obtained when a graph was traced between % reduction and the concentration.

The **IC_{50}** value of all the Fruit extracts **(DCM, Methanol and Water)** have been determined and the corresponding values have been found to be **(40, 38 and 30 µg/ml**) respectively. Similarly the **IC_{50}** value of all the Leaf extracts **(DCM, Methanol and Water)** have been determined and the corresponding values have been found to be **(55, 47, 37 µg/ml**) respectively. Also the **IC_{50}** value of all the Root extracts **(DCM, Methanol and Water)** have been determined and the corresponding values have been found to be **(53, 50, 48 µg/ml**) respectively. From the above results it could be concluded that the **IC_{50}** value decreases correspondingly with the increase in the polarity of the

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solvent. Less is the **IC_{50}** value more is the free radical scavenging power of the concerned plant extract. Among all the savants the water extracts were found to be more potent in inhibiting the free radicals followed by methanol extracts and least activity was noticed for the DCM extracts.

The ascorbic acid exhibited the lowest **IC_{50}** value (26.0µg/ml) with the highest antioxidant potential in relation to all the extracts of fruit, Leaf and Root extracts of ***Hippophae rhamnoides***.

IV. CONCLUSION

The study entitled "Anti-oxidant Properties of ***Hippophae rhamnoides*** showed the comprehensive results as indicated in the representative tables. As per the phytochemical analysis showed that the ***Hippophae rhamnoides*** is rich in almost all types of secondary metabolites which are essential for the continuity of life , because these phytochemicals in addition to their normal physiological role, they boost up the all biological systems which are inter related which each other.. The plant showed a comprehensive anti oxidant property. All types of diseases/ disability which do come across daily life are because of the accumulation of free radicals in the body of humans. Almost all types of diseases do get originated due to the excessive deposition of free radicals. So it becomes necessary to remove these free radicals from the body, because free radicals have a characteristic feature that one free radical leads to the generation of other free radical that is they undergo a chain reaction. This process continuously progresses with the progress of time leading to the generation of diseases or disorders, and also it goes speedily. There is only way to stop this process is the interaction between two free radicals because a radical is a species having only an unpaired electron. So the neutralization of free radicals becomes must. Which is possible either due to the donation or acceptance of that electron, which is present over those free radicals? In a body this donation and acceptance is an ongoing phenomena due to due free radicals gets generated. To overcome this problem there are some aromatic phytochemicals in the form of phenols mostly present in the plants. Those phytochemicals which do process this characteristic feature are known as anti oxidants. As per this mushroom is taken into consideration it showed a healthy result as an anti oxidant, so showed so consumed for a healthy life.

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