Pharmacognostical and Antioxidant Activity of Stem and Root of *Capparis Decidua*

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ABSTRACT :
Herbal Medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. According to the WHO there are 21,000 plant species listed as being medicinally used as plant drugs. Between 70 - 90 % of these are commercially obtained by collecting the drugs in the natural habitat. Capparaceae is a family related to the Cleomaceae and as well as to the Cruciferae family. It is place in the order Brassicales and is commonly known as the caper family. Members of this family contain thioglucosides (known as glucosinolates) which release isothiocyanates (mustard oils) when the plants are damaged. Typically the plants yield methyl isothiocyanate from methyl glucosinolate (otherwise known as glucocapparin). Many of the Capparis species were reported to possess antiinflammatory, antimicrobial, analgesic, anthelmintic and hepatoprotective activities. Believing that the objective of 'Pharmacognostical study and exploration of antioxidant activity of Capparis decidua L. has been selected for the screening of a possible antioxidant effect that the plant might posses.
Key Words: Herbal medicine WHO Capparaceae glucosinolates Cleomaceae isothiocyanates Glucocapparin isothiocyanate, Anthelmintics, Antioxidant & Anticonvulsant.

I. INTRODUCTION

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. In USA, herbal drugs are currently sold in health food stores with a turnover of about $ 4 billion in 1996 which is anticipated to double by the turn of the century1. In India, the herbal drug market is about $ one billion and the export of plant-based crude drugs is around $ 80 million. Herbal medicines also find market as nutraceuticals (health foods) whose current market is estimated at about $ 80–250 billion in USA and also in Europe (Kamboj V.P. 2000). The salient features of WHO guidelines are: (i) Quality assessment: Crude plant material; Plant preparation; Finished product. (ii) Stability: Shelf life. (iii) Safety assessment: Documentation of safety based on experience or/and; Toxicology studies. (iv) Assessment of efficacy: Documented evidence of traditional use or/and; Activity determination (animals, human)(WHO Geneva 1991).

II. PLANT PROFILE

Botanical Description:

Capparis decidua is a small branched tree or shrub of arid regions. It bears a mass of slender, leafless branches, the small caduceus leaves being found only on young shoots.It rarely exceeds a height of 5 meters (15 feet).

Synonyms:

Hindi--*Karel, Kachra,* ***English***--*Caper,* ***Gujarati***--*Kari, Karer, Kanthar, Kerado.* ***Sanskrit*** --*Kareera,* ***Tamil***-*Karyal*

Geographical Distribution:

It is grown mainly in The desert as Sind, Baluchistan, Asia, Africa, Pakistan and India. In India, it is grown in the West of Rajasthan, Gujarat, Panjab(Kiritikar and Basu, 1918).

Plant Description:

Kingdom :Plantae
Subkingdom :Viridaeplantae
Phylum :Tracheophyta
Subphylum :Euphyllophytina
Class :Magnoliopsida
Subclass :Dilleniidae
Superorder :Violanae

Order :Brassicales
Suborder :Capparineae
Family :Capparidaceae
Subfamily :Capparoideae
Genus :Capparis
Species :Capparis decidua

Root	Stem
 (A)	 (B)
Fruit	**Flower**
 (C)	 (D)

Pharmacological uses of *Capparis deciduas:*

Name of *Capparis species*	Plant part	Pharmacological Uses
Capparis deciduas	Stem Powder	Asthma, cough, rheumatism, analgesic diaphoretic, alexeterie, hypoglycaemic &antidiabetic agent, in lowering oxidative stress in diabetes.
	Bark pout	Anthelmintic, constipative, purgative.
	Root bark	Diuretic, paralysis, enlarge, spleen and tubercular gland in rheumatism, expectorant, analgesic reductionof triglycerides, lipids, phospholipid in plasma.

Phytochemical Information of *Capparis deciduas*

Phytochemical investigation of *Capparis decidua* L. has different type of chemical constituents such as alkaloids, glycosides, carbohydrate, tannins, coumarins, steroids, flavanoids. This is evidenced by the growing number of work done in the recent years.

TLC of *Capparis Decidua L. :* The stationary phase used was TLC Silica gel 60 F_{254} aluminium sheets (Merck, Germany). The sample plant extract was prepared simply by just dissolving the required quantity of the extract in methanol.

Quantitative Estimation of the Phytoconstituents of *Capparis decidua L.*

The presence of tannins and phenols in the ethanolic extract of *C.decidua* was confirmed by preliminary phytochemical screening tests, so the estimation of total phenolic and tannin contents was carried out with the study based on the Folin-Coicalteau calorimetric methods of determination proposed by Singleton et al., 1974.

Estimation of total tannin content

Total tannin estimation was done according to the method proposed by Hagerman, A., et al(2000).

Estimation of total flavonoid content

Determination of total flavonoid content was done using aluminum chloride method which uses rutin as the standard reference compound (kumaran, A., et al, 2006). The method is based on the formation of a flavonoid-aluminum complex having the absorption maxima at 415nm.

Total antioxidant assay

Total antioxidant capacity of *C. decidua* was determined by phosphomolybdenum assay by (Prieto et al, 1999). The assay was performed in triplicate.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of *C.decidua* extract was evaluated by method of Jin et al, (1996).

Nitric oxide scavenging activity

The Nitric oxide scavenging activity of C.decidua extract was evaluated by method of Balakrishnan et al, (2009).

III. EXPERIMENTAL:

Equipments: Test tubes, volumetric flasks, graduated pipettes.

Table 1: Preparation of reagents for activity:

Sr. No	Reagent	Preparation
1	GRIESS reagent	0.665ml H_3PO_4 + 0.25g sulfanilamide + 0.025 g α-naphthyl-ethylane dihydrochloride in 25 ml distilled water.
2	Sodium nitroprusside solution (10 M)	0.065 g in 25 ml phosphate buffer (pH 7.4)
3	Phosphate buffer (pH-7.4) • KH_2PO_4 (0.2 M) • NaOH (0.2M)	2.718 g in 100 ml Distilled water 0.8 g NaOH in 100 ml Distilled water (50 ml 0.2M KH_2PO_4 + 39.1ml 0.2 M NaOH)

Preparation of stock solution:

Preparation of sample: Different concentrations (10, 20, 30, 40, 50, µg/mL) of ethanolic extract, ascorbic acid dissolved in phosphate buffer (pH 7.0).

Procedure:

Incubate stock solution with different concentrations of sample at 25 °C for 150 minutes. Control experiment without the test sample but with equivalent amount of buffer was conducted in an identical manner.

After incubation take 0.5 ml of solution add 0.5 ml of Griess reagent (1% Sulfanilamide, 2% 0-phosphoric acid and 0.1% Napthylethylenediamine dihydrochloride). The absorbance was measured at 535 nm by using UV-Visible Spectrophotometer (Shimadzu 250-1 PC) and percentage of inhibition calculated.

$$\%\ inhibition = \frac{OD\ (blank) - OD\ (test)}{OD\ (blank)} x\ 100$$

Ascorbic acid was used as positive control.

Assay of reducing power

The Reducing activity of C.decidua extract was evaluated by method of Kumar et al, (2011).

***IV. IN VIVO* ANTIOXIDANT ACTIVITY:**

Preparation of sample tissue

After performing anticonvulsant activity all mices including Control group, standard group and treated mice with 300mg/kg extract were sacrificed by decapitation . Whole brain of all mices were dissected out, and immediately stored at -80°C until used.

On the day of experiment brain were weighed accurately and divided into three equal parts each for estimation of LPO, CATALASE and SOD level.

Estimation of lipid peroxidation

Lipid peroxidation in liver was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) using the methods of Nehius and Samuelson (1968).

Estimation of Catalase

Catalase (CAT) was estimated by the method of Sinha (1972).

Estimation of superoxide dismutase (SOD) activity

The activity of SOD was assayed by the method of Kakkar et al. (1984).

V. RESULTS:

Morphological characterization:

Leaves: The new flush of leaves appears in November-January. Caduceus, linear, 4-20 mm long, 1-3 mm broad, often spine-tipped, subsessile; stipular spines 1-6 mm long straight or slightly curved, yellow or brown.

Flowers: Red conspicuous flowers appear in March-April and August-September and ripe May and October. Inflorescence few to many flowered ebracteate corymbs on short lateral shoots.Flowers 1-2 cm across on 1-1.5 cm long slender pedicel, usually brick red.

Sepals: Petaloid, usually 5-8 mm long, 3-5 mm broad, ovate-oblong, upper one distinetlysaccate, often with floccose-ciliate margins.

Petals: Petals about as long as the sepals, puberulous, upper pair slightly larger and hidden in the saccate sepal.

Stamens: Generally 10-15, about 10-20 mm long, often red in colour.

Gynophores: 10-15 mm long.

Ovary: Ovary about 2 mm in diameter.

Fruit: Fruit globose, 10-16 mm in diameter, slightly beaked, glabrous smooth, deep red when ripe and with thin pericarpl seeds reinform, 2-5 mm in diameter.

Root: Hardtap root, long cylindrical and thick, secondary and tertiary roots present, yellowish brown in colour.
The morphological studies revealed that the stem was erect, woody, profusely branched, cylindrical, glabrous, solid and green in colour with no odour and bitter taste. The inner wood is yellowish brown in colour. Root is Tap and branched.

Transverse section of root:

The transverse section of the *Capparis decidua* root was taken free handed. The root which is hard was dipped in boiling water or mixture of alcohol and glycerine (1:1) in order to moisten and to make them soft, stained and mounted with glycerin in a slide for observation. The epidermis constitutes of a thick cuticle and was formed by few layers of cork cells. Below the epidermis was the ground tissue (cortex region) made of several layers of rounded collenchymatous cells. The cortex also constitutes of sclerenchymatous cell layers that was made up of sclerenchymatous fibres when viewed in longitudinal section. Well distinct medullary rays were seen.

Powder study:

The root powder is yellowish brown in colour, pleasant odour and slightly bitter in taste. The powders were then visualized under a pale background using a Olympus ch 20 i microscope having the eyepiece 100 X and 45 X magnification power. On microscopical examination the powder showed following structures upon staining with Phloroglucinol-Hcl dye

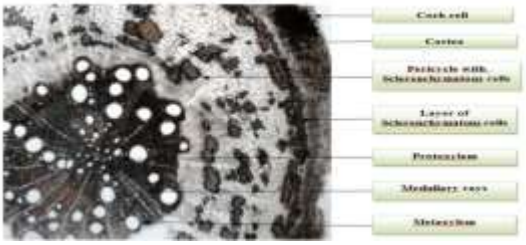


Fig 1: Transverse section of *capparis decidua* root.

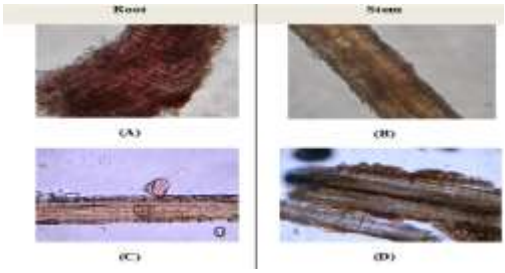


Fig 2: Powder study of *capparis decidua* root.

(A)	Scelerenchymatous cells from root
(B)	Scelerenchymatous cells from stem powder
(C)	Xylem vessels from root powder
(D)	Xylem vessels from stem powder

Physiochemical parameters:

Table 2: Percentage of Foreign matter:

Sr. No	Weight. of the crude drug with foreign matter (in gm)	Weight of crude drug without the foreign matter (in gm)	Percentage weight of foreign matter present per 100gm of air-dried sample.
1	100 gm	96.40	3.60 %
2	100 gm	98.90	1.10 %
3	100 gm	95.50	4.5 %
			Avg. : **3.066 %**

Determination of extractive values (WHO 2002).

Hot Water Soluble Extractable Matter:

Table 3: Percentage of Hot water Soluble Extractive Matter of *Capparis deciduas L.* root

Sr. No	Weight of drug taken (in gm) (W_1)	Weight of Water soluble extractable matter (in gm) (W_2)	Percentage of Water Soluble extractable matter [(W_2/W_1) x 100]
1	4 gm	0.11	2.75 %
2	4 gm	0.10	2.5 %
3	4 gm	0.12	3.0 %
			Avg. : **2.75 %**

Cold Water soluble Extractable Matter:

Table 4: Percentage of Cold Water Soluble Extractive Matter of *Capparis decidua L.* root

S.No.	Weight of drug taken (in gm) (W_1)	Weight of Water soluble extractable matter (in gm) (W_2)	Percentage of Water Soluble extractable matter [(W_2/W_1) x 100]
1	4 gm	0.10	2.5%
2	4 gm	0.12	3.0%
3	4 gm	0.10	2.5%
			Avg. : **2.66 %**

Fluorescence Powder Drug Analysis of *Capparis deciduas:*

The results for the fluorescence powder drug analysis of root is shown below in observation table.

Table 5 : Florescence powder drug analysis of *Capparis deciduas* L. Root

S. No.	Powder + Reagent	Fluorescence in daylight	Fluorescence under UV light	
			254nm	365nm
1.	Dry Powder	Khaki	Khaki	Khaki
2.	Powder+ 1N NaOH in water	Gold	NF	Snow
3.	Powder + 1N NaOH in methanol	Beige	NF	NF
4.	Powder + Glacial acetic acid	Light Yellow	Yellow	Gold
5.	Powder + 1N H_2SO_4	Lightgolden rod yellow	Beige	Beige
6.	Powder + 25% Ammonia	Khaki	Yellow	Gold
7.	Powder + HCl in 1N water	Floral White	Light Cyan	Ivory
8.	Powder + 1N HNO_3	Yellow	Gold	Gold
9.	Powder + Iodine solution	Dark red	Black	Black
10.	Powder + 1N HCl in Methanol	Navajo White	NF	Yellow green
11.	Powder + Picric acid saturated	Yellow	NF	NF
12.	Powder + 50% KOH	Yellow	NF	Old Laoe

Table: 6 Phytochemical screening of Ethanolic extract of *Capparis Decidua* stem and root.

Tests	Aerial Part	Underground Part
Alkaloid	+	+
Glycosides	+	_
Carbohydrate	+	_
Tannins & Phenolics	+	+
Coumarins	_	_
Steroids	+	_
Flavonoids	+	+
Saponins	+	+
Proteins	+	_
Mucilage	_	+
Resins	_	_
Amino acid	_	_

Where, (-): Absent (+): Present

Thin Layer Chromatography:

Result

TLC was run on silica gel g stationary phase the best discovered solvent system was Ethyl acetate : Methanol in a ratio of 1:4. Four spots were observed and tried to identify. The Rf value were calculated using following formula.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

The Different Rf values are. 0.61, 0.69, 0.7, 0.88.

Estimation of Total Phenolic Content:

The results for estimation of total phenolic content were shown below. Standard calibration curve was plotted by the use of Absorbance vs Concentration (µg/ml).

Table: 7 Observation table and Standard curve for total phenolic estimation

Conc. (µg/ml)	Absorbance (765nm)
0	0
5	0.612
10	0.726
15	1.052
20	1.398
25	2.001
50	3.723

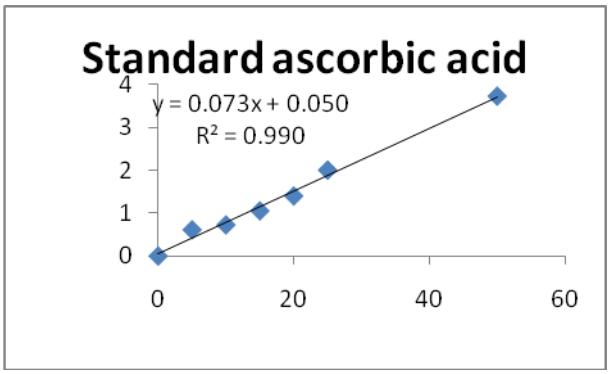


Table: 8 Observation results for ethanolic extracts sample

Ethanolic extract	Absorbance (765 nm)	Total Phenolic content mg/gm plant extract (in GAE)
Ethanolic extract of *C.D.* Underground part	0.409 ± 0.05	51.2mg/gm
Ethanolic extract of *C.D.* Aerial part	0.516 ± 0.05	66.5mg/gm

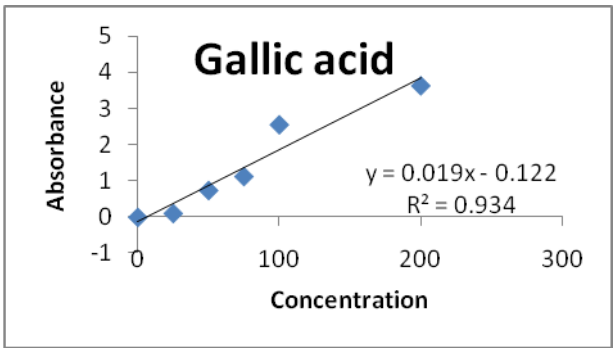
Estimation of Total Tannin Content

Results

The results of observation for the estimation of the total tannin content are shown below. Standard calibration curve was plotted by the use of Absorbance vs Concentration (µg/ml).

Table: 9 Observation table and Standard curve for Total Tannin estimation of sample

Conc (µg/ml)	Absorbance (775nm)
0	0
25	0.105
50	0.742
75	1.129
100	2.561
200	3.636



Estimation of Total Flavonoid Content

Results

The results for the standard and sample observation are shown as follows and the calculation of the total flavonoid

content can be done using the formulae given below.

Table: 10 Observation table for standard

Standard compound	Absorbance (Standard) (415 nm)
Rutin	0.364

Table:11 Observation table for sample

Ethanolic extract	Absorbance (Sample) (415 nm)
C.decidua Aerial Part	0.049
C.decidua Underground Part	0.056

Calculation

The amount of flavonoids in plant extracts in Rutin equivalents (RE) was calculated by the following formula:

$$X = (A \cdot m_o) / (A_o \cdot m)$$

where X is the flavonoid content, mg/g plant extract in RE, A is the absorption of plant extract solution, A_o is the absorption of standard Rutin solution, m is the weight of plant extract, mg and m_o is the weight of Rutin in the solution, mg.

Table:12 Results for total Flavonoid content

Eethanolic extract	Flavonoid content (mg/ml)	Total Flavonoid content mg/gm plant extract (in RE)
C.D underground part	0.00673 mg/ml	0.673mg/gm
C.D. Aerial part	0.00769mg/ml	0.769mg/gm

Comparative *in vitro* Antioxidant Activity of the *Capparis decidua L.* Stem and Root:

Results

The amount of extract needed to inhibit free radical concentration by 50% (IC_{50}), was graphically determined by a linear regression method using MS Windows based Graphpad Instat (Version 3) software.

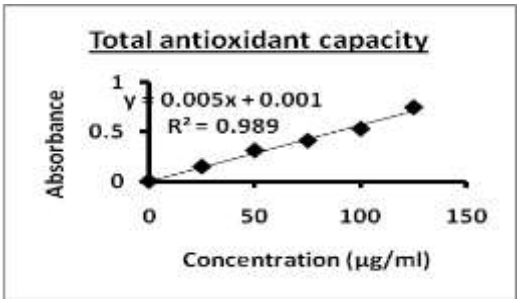
Total antioxidant capacity:

Total antioxidant capacity for aerial part

From the standard graph plotted the results for the samples were interpolated as follows:

Table: 13 Observation table and Standard calibration curve for Ascorbic acid.

Concentration (µg/ml)	Absorbance (695nm)
0	0
25	0.146
50	0.309
75	0.408
100	0.528
125	0.745



Result: The absorbance of ethanolic extracts of *Capparis deciduas L.* aerial part at 695nm was 0.061 ± 0.004 and the total antioxidant capacity observed was 105 µg/ml.

Total antioxidant capacity for underground part:

Result: The absorbance of ethanolic extracts of *Capparis deciduas L.* underground part at 695nm was 0.025 ± 0.001 and the total antioxidant capacity observed was 43.2 µg/ml

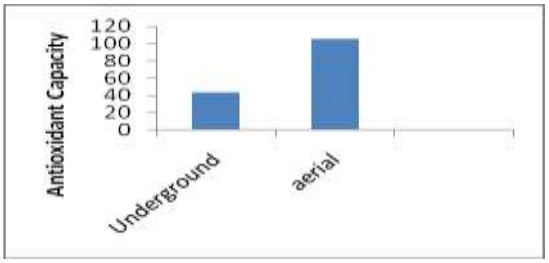


Fig:3 Showing the comparative result of underground and aerial part

Hydroxyl radical scavenging activity:

Table: 14 Observation table for percentage inhibition

Sr. no	Concentration (µg/ml)	Absorbance or percentage inhibition (Mean ± S.E.M)		
		Ascorbic acid	*C. decidua (stem)*	*C. deciduas (root)*
1	0	0	0	0
2	25	56.75±0.54	11.79±1.15	11.38±0.99
3	50	62.15±1.72	32.87±0.58	20.49±1.09
4	75	71.77±1.03	40.14±1.09	26.14±1.72
5	100	77.71±2.27	53.49±1.50	43.95±1.17
6	200	96.25±1.64	54.37 ±0.73	52.48±0.49

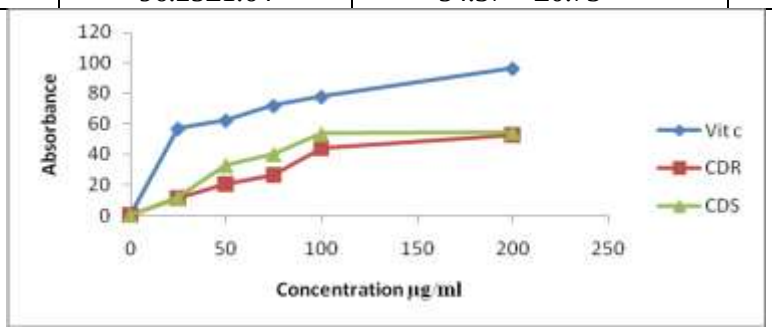


Fig: 4 Showing comparative Hydroxyl radical scavenging activity of *Capparis decidua L.* Aerial and underground part

Table: Results for Percentage Inhibition (IC_{50})

Percentage Inhibition (IC_{50})			
Sample →	**Ascorbic acid**	***C. decidua (stem)***	***C. decidua (root)***
IC_{50} (µg/ml) → ±SEM	**46.89** µg/ml ± 1.316	**141.59** µg/ml ± 1.077	**167.25** µg/ml ± 1.623

Assay for nitric oxide scavenging activity:

Table: 15 Observation table for percentage inhibition

Sr. no.	Concentration (µg/ml)	Absorbance or Percentage inhibition (Mean ± SEM.)		
		Ascorbic acid	*C. decidua (stem)*	*C. decidua (root)*
1	0	0	0	0
2	10	1.963±3.59	2.088±0.57	1.989±0.58
3	20	1.698±7.71	2.939±0.56	2.369±0.53
4	30	1.405±6.68	6.956±1.18	6.045±0.58
5	40	1.28±6.8	10.08±0.53	9.749±0.58
6	50	0.973±10.48	12.658±0.49	10.88±0.61

Results for Percentage Inhibition (IC_{50})

Percentage Inhibition (IC_{50})			
Sample →	**Ascorbic acid**	***C.decidua (stem)***	***C.decidua (root)***
IC_{50} (µg/ml) → ±SEM	**23.44** µg/ml ± 4.24	**194.52** µg/ml ± 8.14	**217.87** µg/ml ± 4.10

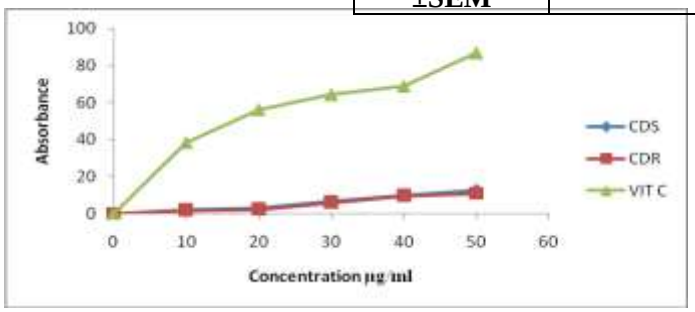


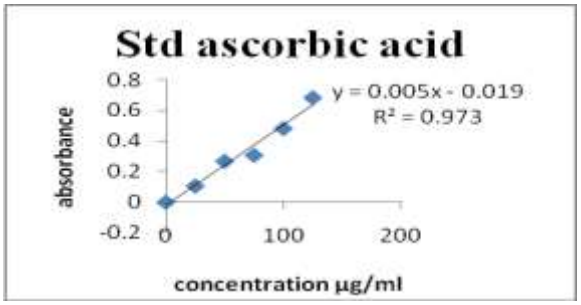
Fig: 5 Showing comparative nitric oxide scavenging property of *Capparis decidua L*. Aerial and underground part

Assay of reducing power :

From the standard graph plotted the results for the samples were interpolated as follows:

Table: 16 Observation table and Standard calibration curve for Ascorbic acid.

Concentration (µg/ml)	Absorbance (695nm)
0	0
25	0.106
50	0.269
75	0.308
100	0.483
125	0.685



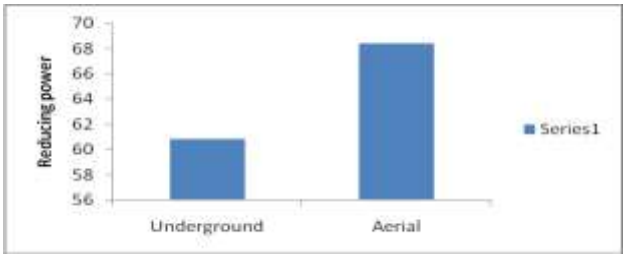


Fig: 6 Showing the comparative result of underground and aerial part

Total reducing capacity for aerial part:

Result: The absorbance of ethanolic extracts of *Capparis deciduas L.* aerial part at 695nm was 0.343 ± 0.04 and the total antioxidant capacity observed was 68.43µg/ml.

Total reducing capacity for underground part:

Result: The absorbance of ethanolic extracts of *Capparis deciduas L.* underground part at 695nm was 0.304 ± 0.05 and the total reducing capacity observed was 60.86µg/ml

VI. DISCUSSION:

The identification of the transverse section is shown by the presence of the different cell layers. The vascular tissues consist of primary phloem, secondary phloem, cambium, primary xylem, secondary xylem. Secondary xylem forms the largest zone. Between the different layers of phloem medullary rays are also present. The powder characters were seen to be solitary and when viewed under the microscope gives the perfect instinct for identification of crude drug. Thus from this we can conclude that fluorescence test gives a brief highlight of how it is applied to differentiate powder and extract of different nature, but still it only provides limited information

regarding the identification of the crude drugs. The phytoconstituents by the use of TLC co-relates with that of the preliminary phytochemical screening. So from this we can conclude that the different classes of phytoconstituents present in the *Capparis decidua* extract shows the confirmation for the presence of the carbohydrate, steroids, tannins and phenolics and flavonoids. By the comparison of above results we can say that the extract of *capparis decidua* of stem and root both part have some antioxidant activity, But *capparis decidua* ethanolic extract of stem posses more antioxidant activity than the root extract. The presence of polyphenols (both phenolic and tannin) in the plant extracts reveals that there might be various activities that can cope up to treat different ailments and disorders.

VII. CONCLUSION:

The present study on Pharmacognostical, antioxidant activity of stem and root of *Capparis decidua* will provide useful information for its identification and pharmacological property. The work concluded in this has followed WHO guidelines (2002), and IHP guidelines (2002) to standardize the *Capparis decidua* root. Extract of aerial portion having more potential as compared to the extract of underground portion in case of antioxidant study. The phytochemical study reveals the presence of phenolics , tannins and flavonoids in the extract of plant parts. To make beneficial use of this plant it needs further study in terms of standardization and quality control at molecular level.

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