



EVALUATION OF CHEMICAL PROFILE AND IN VITRO ANTIOXIDANT STUDY OF DIFFERENT PARTS OF ADHAHAPUSHPI (TRICHODESMA INDICUM LINN R.BR.)

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Abstract

Evidence suggests that the plants are considered a good source for chemoprevention and cancer therapy due to the high concentration and wide variety of antioxidants such as vitamin A, vitamin C, polyphenols, (–)Epigallocatechin 3-gallate, flavonoids, polyphenols, gallic acid, glycosides, epicatechin, quercetin. *Adhahapushpi*(*TrichodesmaindicumLinn.RB.*) Belonging to Boraginaceae family is traditionally used as anticancer to treat breast cancer in Chattisagharha. It suggests its antioxidant content. The present study is conducted to evaluate antioxidant activity and chemical composition of different parts of Adhahapushpi (*Trichodesma indicum Linn R.Br.*). DPPH essay is widely used methodology to determine antioxidant capacity which measures the reducing ability of antioxidants toward the DPPH radical. Ethanol, Methanol and Water extracts of different parts of Adhahapushpi are tested for antioxidant activity using in vitro model- DPPH essay. Result showed that the total phenolic content(Gallic acid) ranged between 29.50 to 73.21 mg/gm extract and total flavanoid (Quercitin) ranged between 2.63 to 18.69 mg/gm extract. Inhibition rate in DPPH antioxidant essay ranged between 63.9 to 82.1%. From the study it is concluded that the different parts of *Adhahapushpi*(*TrichodesmaindicumLinn.RBr.*) can be a good source of natural antioxidant.

Key words: *Adhahapushpi*, Boraginaceae, , phenolics, flavanoids, gallic acid, quercitin, antioxidant activity DPPH essay.

Introduction

It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential. Increased oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases and antioxidants produced by plants are capable of mitigating ROS- induced oxidative damage.^[1]

Evidence suggests that the plants are considered as good source for chemoprevention and cancer therapy due to the high concentration and wide variety of antioxidants such as vitamin A, vitamin C, polyphenols, (–)Epigallocatechin 3-gallate, flavonoids, polyphenols, gallic acid, glycosides, epicatechin, quercetin, curcumin, lovastatin, and many other types of compounds with the capability to inhibit the cell proliferation of different cancer cells *in vitro*, such as colon cancer (HT-29, SW48, HCT116), breast (MCF7, MDA), cervix (HeLa, SiHa, Ca-Ski, C33-A), liver (Hep G2), skin (A 431), and many other malignant cells; studies have indicated that antioxidants can be employed efficiently as chemopreventives and as effective inhibitors of cell proliferation, promoting cell apoptosis, and increasing detoxification enzymes, and inhibiting gene expression and scavenger Reactive oxygen species (ROS). Thus, many researches are conducted with different types of natural antioxidants to find out effective drug that can inhibit the development of cancer both *in vitro* as well as *in vivo*, because these compounds have exhibited high potential for use equally in the treatment of this disease as well as chemoprotective agents.^[2]

Adhahapushpi(*Trichodesma indicum*Linn.*R.B.*).is a hispid herb belonging to Boraginaceae family, is an important folklore medicinal herb used for different ailments such as urinary disorders, diarrhoea, dysentery, dysmenorrhoea, joint disorders,snakebite,tumours,breastcancer and much more diseases by both Tribal and AYUSH systems of Medicine.^[3] Previously the arial part of the plant was screened for antioxidant activity.^[4] The current study is undertaken to study total phenolic and flavanoids present in different parts viz, Root, Stem, Leaf and Fruit as well as their antioxidant property. The Alcohol, Methanol and Water extracts were tested for antioxidant activity using *in vitro* DPPH method.

Material and Methods

Collection of the Plant material: The plant *T. indicum* Linn, R.Br is collected from Dhanvantari vana of S.G.V.Ayurvedic Medical college Bailhongal and is authenticated by RCMR Belganvi, voucher specimen PHD/SDB/AAMC-11 was deposited to the Department of Dravyaguna vignana, Alvas Ayurvedic Medical College Moodbidri.

Preparation of Extraction:

Extraction procedure using organic solvents by cold extraction method^{[5]:}

About 4.0g of Root, leaf, stem and fruit of plant powder of the *Trichodesma indicum* Linn. *R. Br.* is placed separately in an accurately weighed, glass stoppered conical flask. For estimation of hot water-extractable matter, 100ml of distilled water was added to the flask and total weight including the flask was weighed with Electronic top loading balance. The contents were shaken well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and boiled gently for 1 hour; cooled and weighed. The flask was readjusted to the original total weight with distilled water and shaken well and filtered rapidly through a dry filter. Then 25 ml of the filtrate was transferred to an accurately weighed Petri dish and placed on a water-bath to evaporated to dryness. Finally, it was dried at 105°C for 6 hours in an oven, cooled in a desiccator for 30 minutes, and weighed without delay. Same procedure was followed using ethanol and methanol to determine ethanol and methanol extractable matter. The extractable matter was calculated as the content of in mg per g of air-dried material.

Quantification of total flavonoids^[6]

Total flavonoid content of plant was determined by Aluminium chloride colorimetric method, quercetin was used as standard.

Brief protocol:

Quercetin was used to make the calibration curve by dissolved in methanol/Ethanol and then diluted to 6.25- 200 µg/ml of serial concentrations. Stock Solution of Extracts(1mg/ml) was prepared with methanol/Ethanol.

Reaction Solutions of 10 ml contained: Sample extract stock solution / (quercetin standard) to which Methanol, 10% Aluminium Chloride, 1M Potassium Acetate solution and distilled water and were added and mixed well. Sample blank was prepared in similar way by replacing Aluminium chloride with distilled water.

Sample and standard absorbance was measured at 420 nm with a Shimadzu UV-1800 spectrophotometer. Calibration curve using ABSORBANCE vs CONCENTRATION of Quercetin standard ($R_2 = 0.997$) was prepared and the concentration of total flavonoid in the sample was determined by using a slope equation that was obtained from the standard graph and results for total flavonoids was expressed as mg of quercetin equivalent /gm dried extract.

Determination of Total Phenols^[7]:

Total phenols content of plant was determined by with the Folin- Ciocalteu colorimetric method, Gallic acid was used as standard.

Brief protocol:

Gallic acid was used to make the calibration curve by dissolved it in methanol and then diluted to 6.25 - 100 µg/ml of serial concentrations. Stock Solution of Extracts (1mg/ml) was also prepared with methanol.

Reaction Solutions of 10ml contained: Sample extract stock solution / (Gallic acid standard) was mixed with 10 fold dilute Folin-Ciocalteu reagent and 7.5% sodium carbonate. The tubes were covered with Parafilm and allowed to stand for 30 minutes at room temperature then the absorbance was read at 760 nm. Blank was prepared in similar way by replacing sample or standard with methanol. Sample and standard absorbance was measured at 760 nm with a Shimadzu UV-1800 spectrophotometer.

Calibration curve was prepared using ABSORBANCE vs CONCENTRATION of gallic acid standard($R_2 = 0.999$). The concentration of total flavonoid in the sample was determined by using a slope equation that was obtained from the standard graph and results for total phenols was expressed as mg of gallic acid equivalent /gm dried extract. A calibration and the results were expressed as milligram of tannic acid equivalents/gram fresh weight i.e. mg TAE/g FW.

Antioxidant activity:**DPPH assay (2,2-diphenyl-1-picryl-hydrazyl-hydrate)^[8]****Principle:**

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. It is rapid, simple and inexpensive and widely used method to measure ability of compounds to act as free radical scavengers. It is free scavenging activity of compound. DPPH is stable free radical in ethanolic solution. In its oxidized form DPPH radicals has higher absorbance at 520 nm. In this assay percentage inhibition of DPPH is calculated with standard ascorbic acid. It is reduced in the presence of an antioxidant molecule, giving rise to colourless ethanolic solution. It is stable at room temperature. The amount of reduced DPPH radicals is proportional to the amount of oxidized antioxidants.

Reagents:

0.2 mM DPPH

0.1 M Tris HCL (pH 7.4)

Preparation of extract: Weigh 10mg of extract (the sample given for study) and dissolve in 1ml of DMSO.

Procedure

3 test tubes were taken and labeled as Blank, control and test. Reagents were added as follows.

Blank: 600 µl Ethanol + 400 µl Tris HCL

Control: 100 µl Ethanol + 400 µl Tris HCL +500 µl DPPH solution

Test: 100 µl sample + 400 µl Tris HCL + 500 µl DPPH solution

All the tubes were shaken and kept in dark for 30 min. After 30 minutes of incubation, the

Decolourisation of the purple colour was measured at 490nm. The radical scavenging activity was calculated as follows:

Calculation

As = sample O.D. Ac = control O.D.

Inhibition Ratio % = $\frac{Ac - As}{Ac} \times 100$

Statistical Analysis: Statistical analysis was carried out using one-way method ANOVA. Correlations between Total phenolic content(TPC), Total flavanoid content(TFC) and DPPH capacity were determined using Pearson's correlation coefficient (r).

Result :

Different extracts of various parts of Adhahapushpi (*Trichodroma indicum* Linn R.Br.) showed antioxidant effect which is due to the phenolic components such as flavonoids viz. Quercetin, Gallic acid etc.

Table No.1 Total Phenolic contents (Gallic acid):

Type of cold Extract	Leafmg/gm Extract		Rootmg/gm Extract		Stemmg/gm Extract		Fruitmg/gm Extract		ANOVA/Unpaired t Test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Aqueous	43.65	+/- 0.19	56.48	+/- 0.94	43.40	+/- 0.36	33.33	+/- 0.57	F=801.08 P<0.001*
Methanol	73.21	+/- 0.38	-	-	-	-	29.50	0.56	t=111.07 P<0.001*
Alcohol	50.48	+/- 0.47	34.70	+/- 0.25	37.95	+/- 0.25	76.83	+/- 0.19	F=11512 P<0.001*

***:Statistically significant n=3**

Table No.2 Total Flavanoid contents (Quercetin acid) in mg/gm Extract:

Type of cold Extract	Leafmg/gm Extract		Rootmg/gm Extract		Stemmg/gm Extract		Fruitmg/gm Extract		ANOVA/Unpaired t Test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Aqueous	15.26	+/- 0.65	4.49	+/- 0.10	7.62	+/- 0.45	17.31	+/- 0.63	F=432 P<0.001*
Methanol	16.41	+/- 0.91	0.40	0.28	-	-	-	-	t=29.128 P<0.002*
Alcohol	18.69	+/- 0.36	2.63	+/- 0.54	3.41	+/- 0.21	14.78	+/- 0.58	F=978.02 P<0.001*

***:Statistically significant n=3**

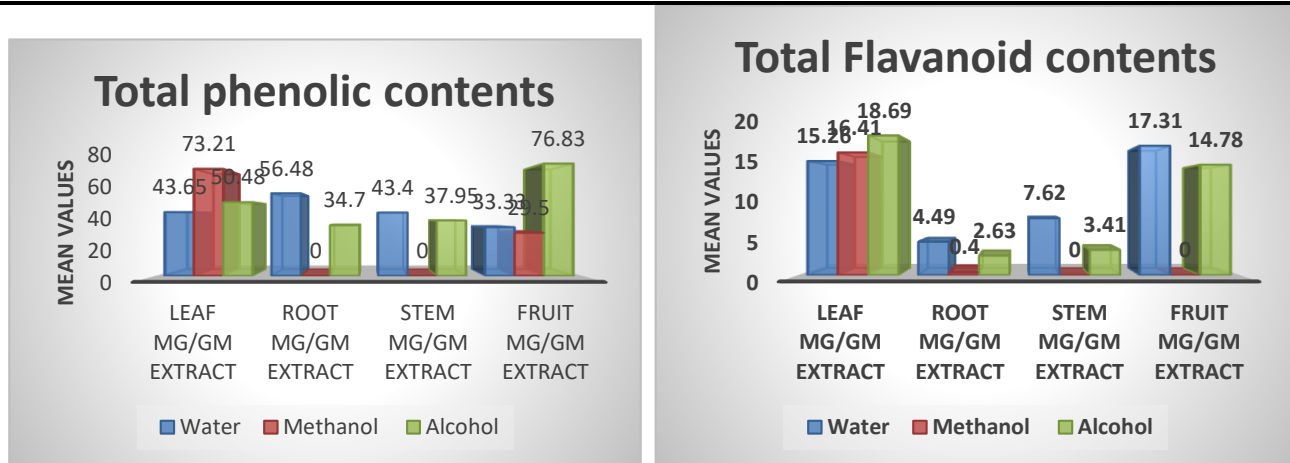


Fig.No.1 A. Total Phenolic contents (Gallic acid) B. Total Flavanoid contents(Quercitin)

Table no.3 Antioxidant activity of different parts of Adhahapushpi(Trichodesma indicum Linn.R.Br.)

Sample	Alcohol	Methanol	Aqueous
Root	72.3 +/-0.01	70.4 +/-0.06	72.3 +/-0.01
Leaf	77.2 +/-0.07	58.8 +/-1.05	15.0 +/-0.26
Stem	72.6 +/-0.14	ND	75.3 +/-0.05
Fruit	63.9 +/-0.12	71.3 +/-0.08	82.1 +/-0.25

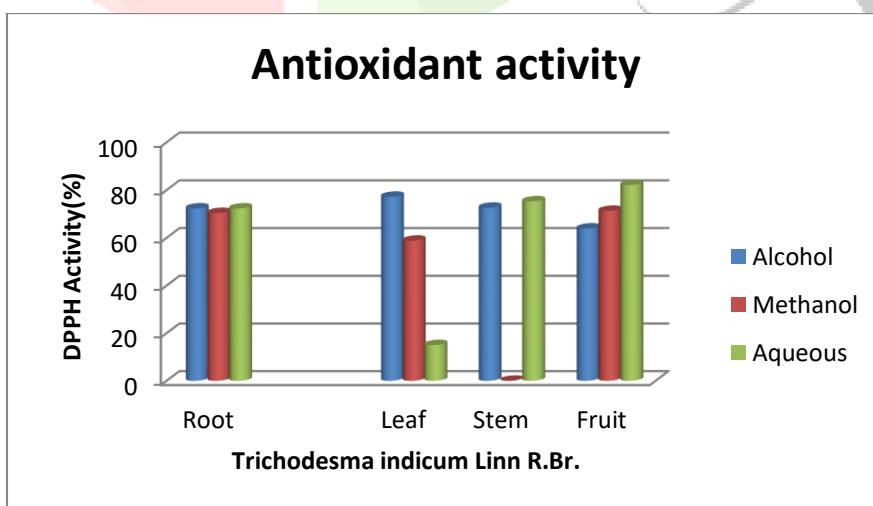


Fig. No. 3 Antioxidant activity of different parts.

Table no.4 Correlation between Total phenolic content (mg TAE/g FW) and DPPH:

Correlation between Total phenolic content (mg TAE/g FW) ^a and DPPH			
Plant part	Correlation coefficient (r)	P value	Remark
Root	r=0.9240	0.2497	Perfect positive correlation. Statistically not significant
Stem	r=0.9965	0.0434*	Perfect positive correlation. Statistically significant
Leaf	r=0.4386	0.7110	Moderate positive correlation. Statistically not significant
Fruit	r=0.786	0.4481	Moderate positive correlation. Statistically not significant

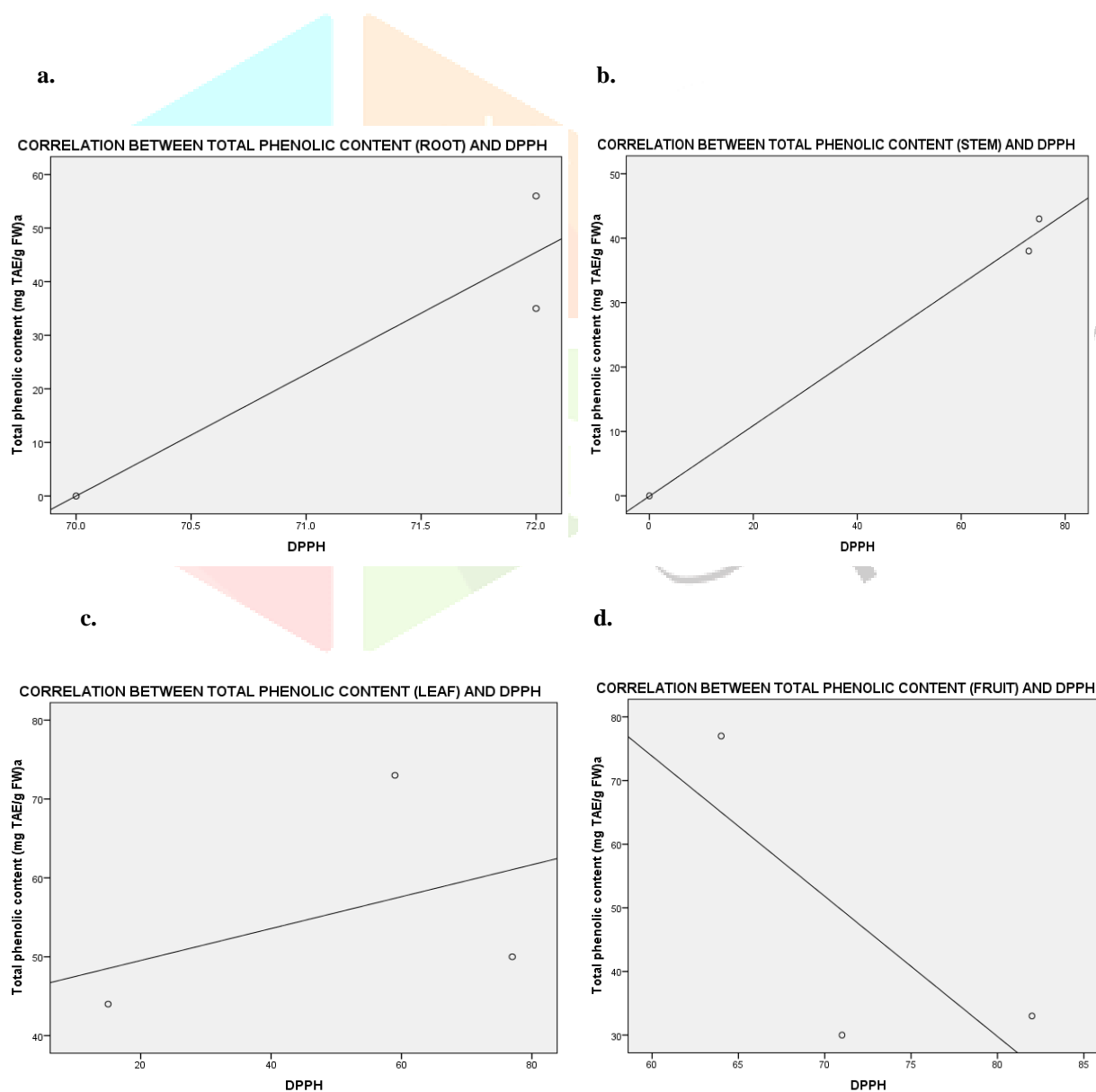
**Fig. No. 4 Correlation between TPC and DPPH of a. Root b. Stem c. Leaf d. Fruit**

Table No. 5 Correlation between Total flavonoid content (mg RE/g FW) and DPPH

Correlation between Total flavonoid content (mg TAE/g FW) ^a and DPPH			
Plant part	Correlation coefficient (r)	P value	Remark
Root	r=0.8909	0.3001	Moderate positive correlation. Statistically not significant
Stem	r=0.8512	0.7245	Moderate positive correlation. Statistically not significant
Leaf	0.9133	0.8341	Moderate positive correlation. Statistically not significant
Fruit	0.2408	0.0479*	Mild positive correlation. Statistically significant

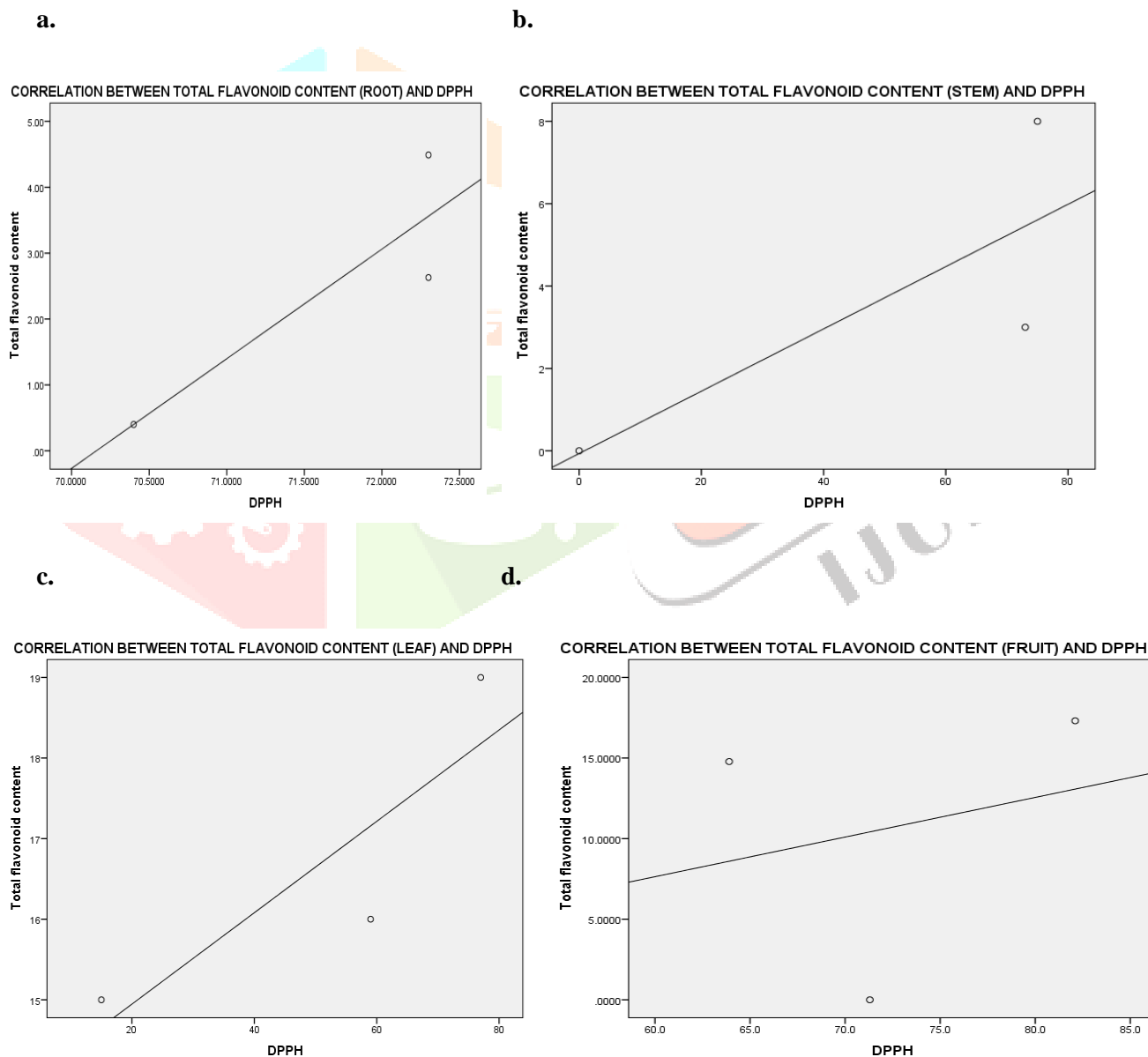


Fig. No. 5 Correlation between TFC and DPPH of a. Root b. Stem c. Leaf d. Fruit
Correlation between Total flavonoid content (mg RE/g FW) and DPPH

Discussion:

The powder of different parts of plant was extracted using different solvents of increasing polarity viz methanol, ethanol and water. The obtained extracts were studied for total phenolics and flavonoids. The extracts showed a notable amount of phenolics and flavonoids (Table no.1 and 2), which varied widely.

Total Phenolics(Gallic acid): Leaf : Methanol extract has higher Gallic acid compared to water and Alcohol extracts. Methanol extract > Alcohol > Aqueous. Root : Gallic acid was not traced out in Methanol extract. Aqueous extract has higher Gallic acid compared to Alcohol extracts. Stem : Gallic acid was not traced out in Methanol extract. Aqueous extract has higher Gallic acid compared to Alcohol extracts. Fruit : Alcohol extract has higher Gallic acid compared to Aqueous and Methanol extracts. Alcohol extract > Aqueous > Methanol.

Total Flavanoid (Quercetin acid):

Leaf : Alcohol extract has higher Quercetin compared to water and Methanol extracts. Alcohol extract > Methanol > Aqueous.

Root : Aqueous extract has higher Quercetin compared to Alcohol extracts. Aqueous extract > Alcohol > Methanol.

Stem : Quercetin was not traced out in Methanol extract. Aqueous extract has higher Gallic acid compared to Alcohol extracts.

Fruit : Quercetin was not traced out in Methanol extract. Aqueous extract has higher Gallic acid compared to Alcohol extracts.

In vitro antioxidant activity: The DPPH assay is the most commonly used assay to determine radical scavenging property.

Different extracts of different parts of *Adhahapushpi* (*Trichodesma indicum* Linn R.Br). was subjected for DPPH assay and the percentage of inhibition was noted. Water extract of fruit has the highest inhibition (82.1+/-0.25), followed by Alcohol extract of leaf (77.2+/-0.007) where as methanol extract of stem did not show any scavenging activity and the lowest was recorded for aqueous extract of leaf.

Corelation between Total phenolics, Total flavanoid and Antioxidant activity: From table no 5 and 6 it is evident that there is mild to moderate positive relation between Total phenolics, Total flavanoids and DPPH assay. Total phenolics and DPPH of stem extracts having statistically significant value. Similarly Total flavanoids and DPPH of fruit extracts having statistically significant value.

Conclusion:

The data obtained in the study suggest that all the extracts obtained from the selected plant *Adhahapushpi* (*Trichodesma indicum* Linn R.Br.) has phenolics, flavonoids and antioxidant capacity, although slight variation is there. There is significant correlation between Total phenolics, total flavanoids and Antioxidant capacity so different parts of *Adhahapushpi* (*Trichodesma indicum* Linn R.Br.) could be considered as a significant source of natural antioxidants.

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Pictorial abstract:

