WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 618-628. Research Article ISSN 2277–7105

PHYTOCHEMICAL ANALYSIS AND ANTI-OXIDANT ACTIVITY OF *NOTHOPODYTES NIMMONIANA* (GRAH.) MABB ROOT AND LEAF

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Article Received on
23 Sep. 2017,
Revised on 13 October 2017,
Accepted on 02 Nov. 2017
DOI: 10.20959/wjpr201715-10042

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ABSTRACT

Oxidative stress in human body contributes to the pathogenesis of many human diseases. Antioxidants are found naturally in plant materials and also in supplements. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic one. *Nothopodytes nimmoniana* (J. Graham) (Icacinaceae), commonly known as “Narakhya” been used for the treatment of cancer in villages. Camptothecin (CPT), a monoterpene indole alkaloid, present in the drug is regarded as one of the most promising anticancer drug of the 21st century. However, it is currently unknown which phyto-constituent is responsible for anticancer activity. Therefore, an attempt is made to fractionate and isolate active ingredient(s) from this plant, Experimentally, first, the root and leaf of *Nothopodytes nimmoniana* were extracted sequentially using hexane, dichloromethane, chloroform, aqueous ethanol (70% ethanol) and water. Next, characterisations were done by quantitative analysis for Total Carbohydrates, Glucose Oxidase, Reducing sugars, total phenols and Antioxidant Assay. The data showed a significant fraction with Antioxidant activity with Chloroform being the most effective fraction.

KEYWORDS: Nothopodytes nimmoniana, phyto-constituent, anti-cancer activity.

INTRODUCTION

Nature has been recognized as a rich source of medicinal compounds for hundreds of years. Nothapodytes nimmoniana (J. Graham) Mabberly (Syn: “Narakya”/ "Kalagur", Family Icacinaceae) is a small tree distributed in Western Peninsula from Konkan Southward i.e.

Nilgiris, Konkan Ghats, broadly the Western Ghats of India, a global biodiversity hot spot. It consists of Camptothecin, a monoterpene indole alkaloid, was first discovered in the Chinese deciduous tree Camptotheca acuminate.[1] It is an important medicinal plant, the major source of a potent alkaloid, namely camptothecin, of a wide spectrum of pharmacological activities like anti-cancer, anti-HIV, antimalarial, antibacterial, anti-oxidant, anti-inflammatory, anti-fungal and also applied in the treatment of anaemia. Camptothecin contains a pentacyclic ring system that includes a pyrroll (3,4-b) quinoline moiety (ring A, B and C). Camptothecin and its derivatives are unique in their ability to inhibit DNA topoisomerase I by stabilizing a covalent reaction termed the cleavable complex which ultimately causes tumor cell death. Topothecan is water-soluble analog of the natural chemical compound camptothecin. Camptothecin is regarded as one of the most promising anticancer drug of the twenty first century.[2] Studies show anti-cancer activities on breast cancer[3], cervical cancer[4], ovarian cancer[5], lung cancer.[6] Hence present study aims at showing the phytocemical analysis followed by antioxidant assay of Notopodytes nimmoniana root and leaf.

MATERIALS AND METHODS

1.1 Collection of Plant Material

Nothopodytes nimmoniana plant is collected from sakleshpur(Western ghats), Hassan district, Karnataka. The samples included Root and leaf. The samples were authenticated by expert and voucher specimen deposited in Department of Dravyaguna JSS Ayurveda Medical College Mysuru. Further, the samples were dried under shade, sieved through 40mm stainless steel and stored in air tight container and taken for further analysis.

1.2 Preparation of crude extract

Crude Extract of Nothopodytes nimmoniana is prepared by taking 20gm dried powder of each i.e Root and Leaf Sample. The extraction process were carried out in a sequential manner from non-polar to polar solvents starting from Hexane, Di-chloro methane, chloroform, Hydroalcohol(70% ethanol) and water. Starting with 100ml of hexane solvent, 20gms of root and leaf *of Nothopodytes nimmoniana* is tranferred to each conical flask and kept in multispin(Spinwin-Colgate company) at 1010 rpm for 3hrs. After 3 hrs, the samples are transferred to 50ml falcun tubes and kept in centrifuge for 10 min at 5000rpm. The supernatant is filtered using filter paper and the remenant is transferred back to conial flask and 100ml of fresh hexane is added to it and kept in multispin for 3hrs at 1010rpm. The total procedure is repeated for 3 times. The supernatant is collected and stored in a wide mouth

glass container for drying. After drying of sample the next solvent is added and the procedure is repeated for 3 times and with all the solvents. Next, the extract is kept in fume hood to dry. After complete drying of sample, the yield extracts are weighed and stored.

Remenant portion of the sample is dried with Pet Ether and dried completely and the residue is weighed and stored in an air tight container.

RESULTS AND DISCUSSION

Yield of different fractions collected from *Nothopodytes nimmoniana* root and leaf.

1. ROOT

Extractant	Material weight (In grams)/20grams	% Yield
Hexane	0.916	4.5
Dichloromethane (DCM)	2.403	12.015
Chloroform	0.208	1.04
Aqueous ethanol (70%)	2.161	10.8
Water	1.511	7.6
Insoluble residue	9.107	45.5
Total	16.306	81.5

Fig No 1- Different fraction of Nothopodytes nimmoniana Root

2. LEAF.

Extractant	Material weight (In grams)/20grams	% Yield
Hexane	0.09	0.45
Dichloromethane (DCM)	0.78	3.90
Chloroform	0.12	0.60
Aqueous ethanol (70%)	0.73	3.65
Water	0.18	0.90
Insoluble residue	13.50	67.5
Total	15.40	77.00

Fig No 1- Different fraction of Nothopodytes nimmoniana Leaf

2.3 Characterisation of each extract

Preliminary quantitative phytochemical analysis for Total Carbohydrates, Glucose oxidase, reducing Sugars, total phenolics are made by following standard procedures. In vitro antioxidant properties were evaluated by assessing FRAP and DPPH.

2.31 ESTIMATION OF TOTAL CARBOHYDRATE BY PHENOL-SULPHURIC ACID METHOD[4]: In this method the concentrated H_2SO_4 reacts with phenol to produce a yellow gold color & absorbance measured at 490nm.

2.32 ESTIMATION OF GLUCOSE BY GLUCOSE OXIDASE METHOD[5]: In this method the glucose oxidase reaction precipitates during oxidation and condensation with phenol and 4-Amino anti-pyrine in the presence of horse reddish peroxidation to produce Red quinine dye and absorbance measured at 505nm.

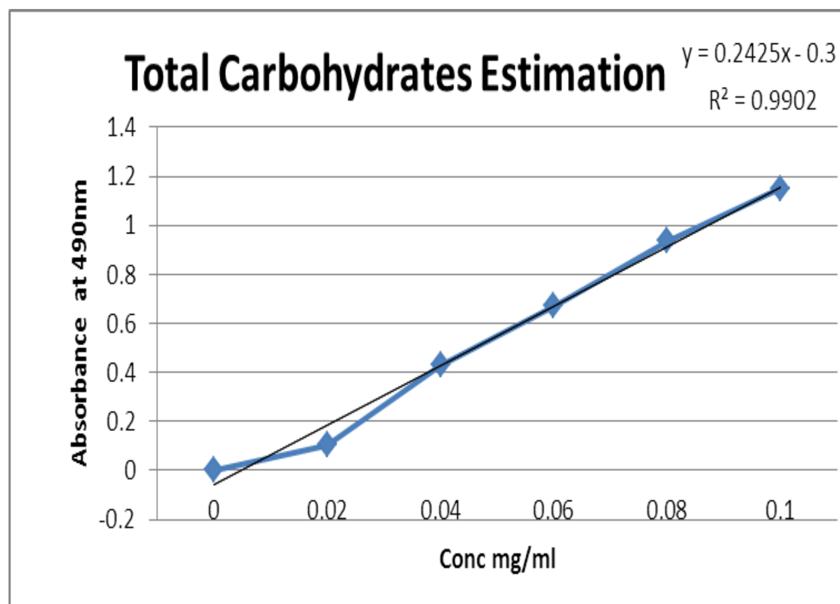
2.33 ESTIMATION OF FREE REDUCING SUGAR BY 3,5 DINITRO SALICYCLIC ACID METHOD[6]: In this method sugar acts as reducing agent that reduces 3,5 DNSA under alkaline medium to form an orange colored product and absorbance at 520nm.

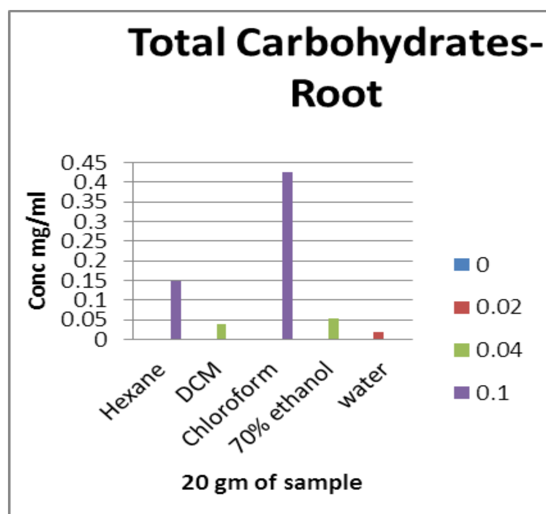
2.34 ESTIMATION OF TOTAL PHENOL BY GALLIC ACID METHOD/ FC METHOD[7]: Phenols reacts with phosphomolybidic acid present in Folin-Ciocaltease in alkaline medium to give a coloured complex which can be read at 765nm. The colour developed is directly proportional to the phenol content

Anti-oxidant Assay

2.35 Estimation of Antioxidant activity by FRAP method(Ferrous reducing activity of plasma)[8]: The ferric reducing ability of the plasma is the measure of anti-oxidant activity which is based on the reduction of the Ferric-TPTZ (Fe^{+3} –TPTZ) complex to Ferrous-TPTZ (Fe^{+2} –TPTZ) complex at lower pH of 3.6, in the presence of an antioxidant. The blue colour formed is directly proportional to the anti-oxidant activity read at 593 nm. Ferrous sulphate is used as a standard. From the standard values, the FRAP units of the sample are calculated.

2.35 Estimation of Antioxidant activity by DPPH Method[9]: DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical, due to the delocalization of the spare electron on the whole molecule. Thus, DPPH does not dimerize, as happens with most free radicals. The delocalisation on the DPPH molecule determines the occurence of a purple colour, with an absorbtion band with a maximum around 520nm. When DPPH reacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, accompanied by the disappearance of the violet colour. Therefore, the absorbance diminution depends linearly on the antioxidant concentration.[10-12]





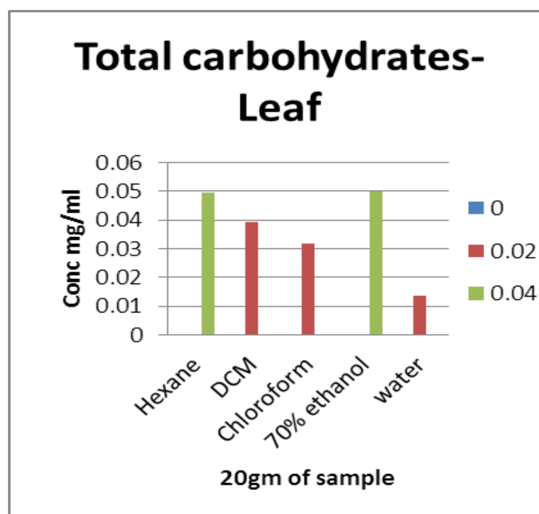
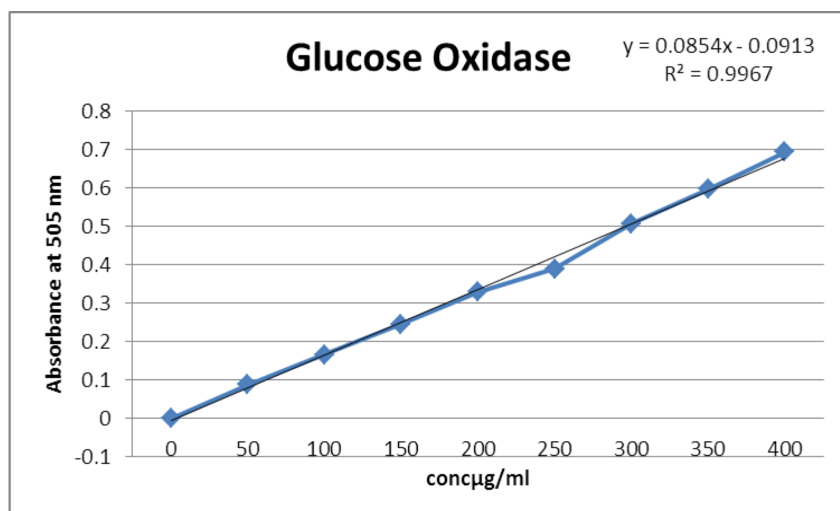


Fig 1: Total carbohydrates by Phenol Sulphuric Acid, absorbance at 490nm, Fig 2 and Fig 3: Total Carbohydrates root and leaf sample.



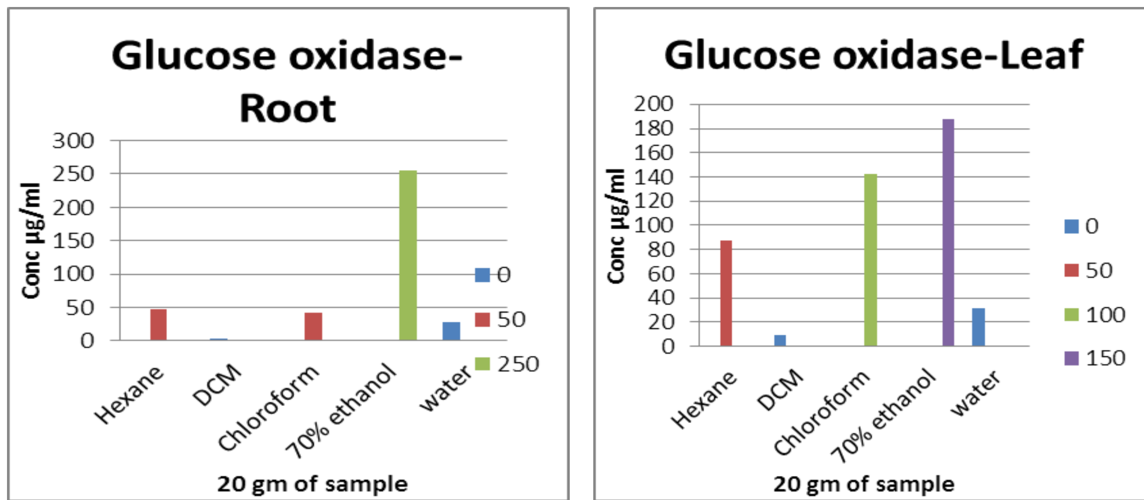
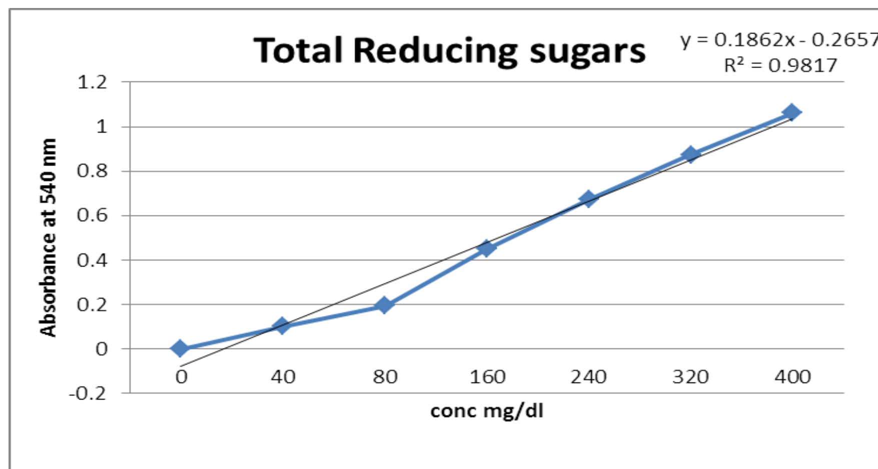


Fig 4: To Estimate plasma glucose by Total Glucose oxidase method and absorbance at 505nm. Fig 5 and 6: estimation of glucose with root and leaf sample.



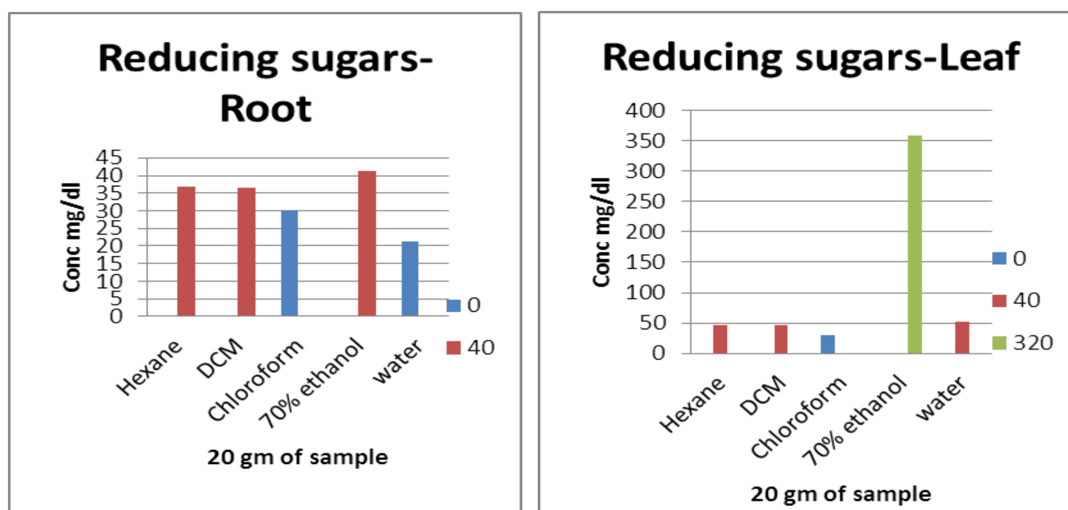
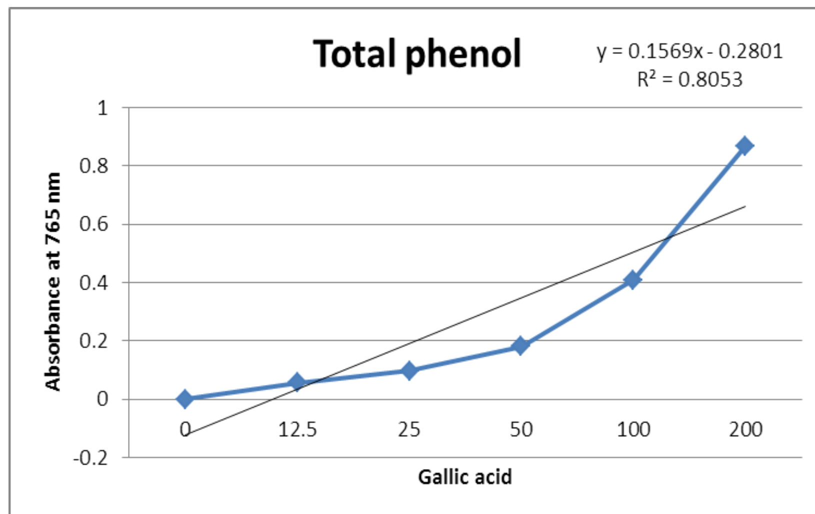
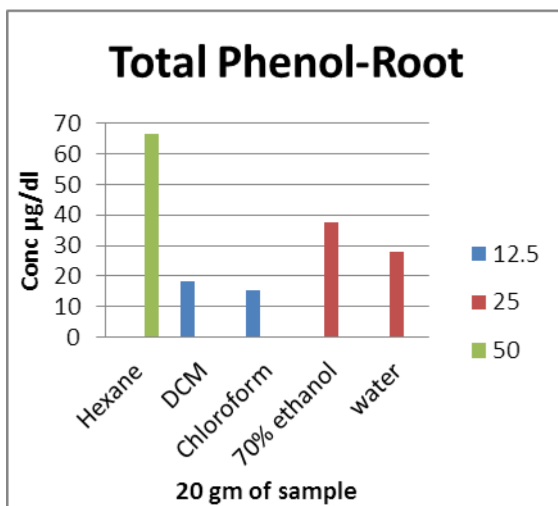


Fig 7: To estimate the amount of free reducing sugars by DNSA(Dinitro Salicyclic Acid Method). Fig 8 & 9: Reducing sugar with root and leaf sample.





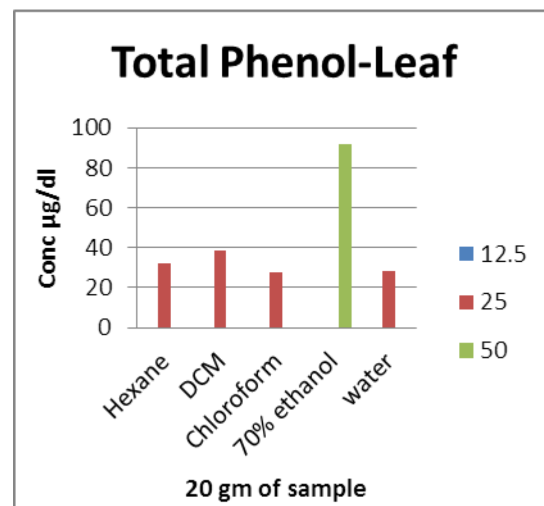


Fig 10: To determine phenolic acid content by FC method, Fig 11 and 12: Total phenols showing Root and Leaf extract.

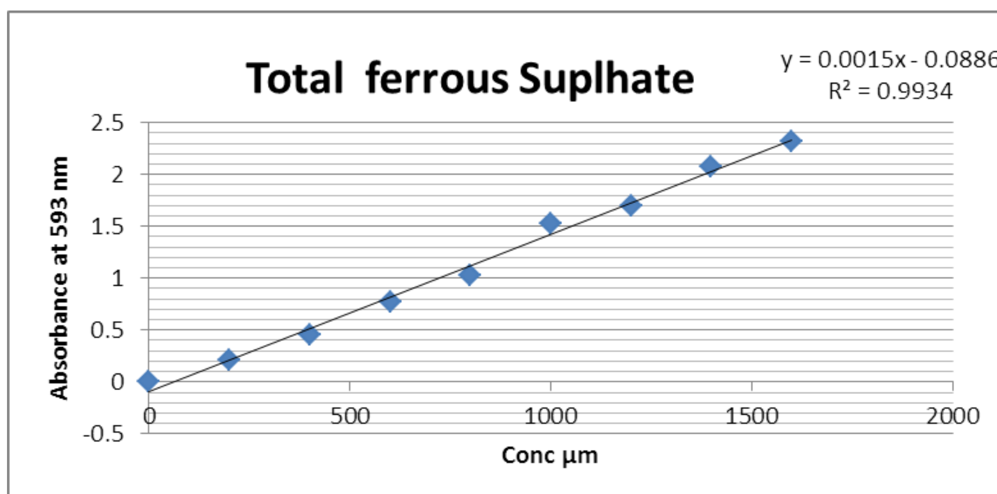


Fig 13, Fig 14 and 15: Estimation of ferric reducing ability of the plasma based on the reduction of ferrous sulphate.

FRAP ASSAY(Root)

Concentration	Hexane	DCM	Chloroform	70% ethanol	water
Mg/mL TP*					
100µg	313	11.7	761.5	835	1358
50 µg	256	10.6	457	577	831
25 µg	247	9.1	296	403	545
12.5 µg	233	8.25	166.5	337	353
6.2µg	180	8	129	249.5	286

***Total Phenol**

FRAP ASSAY(Leaf)

Concentration					
Mg/mL TP*	Hexane	DCM	Chloroform	70% ethanol	water
100µg	737.5	100.25	952.5	1986.5	2938
50 µg	716.5	54.85	892	1216	1698
25 µg	469.5	26.55	353	743.5	998
12.5 µg	419.5	12.45	193.5	497	573
6.2µg	315	4.4	107.5	401	493

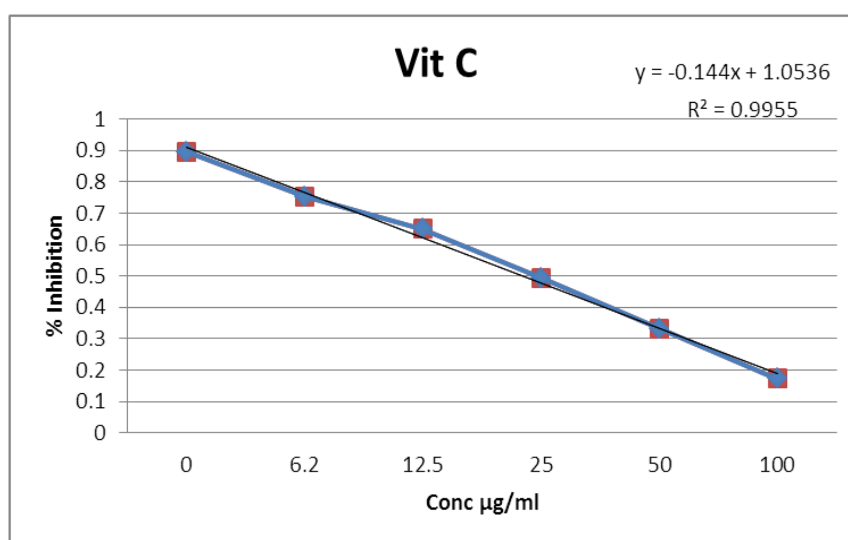


Fig 16, 17 and Fig 18: Estimation of % pf scavenging activity by Vit C with root and leaf extract.

DPPH ASSAY Leaf

Concentration (µg/mL)					
	Hexane	DCM	Chloroform	70% ethanol	water
50µg	16%	66%	91%	86.85%	30.12%
25 µg	16%	51%	91%	74.05%	15.30%
12.5 µg	11%	43%	87%	65.45%	4.60%
6.2 µg	7%	31%	84%	41.15%	0.55%
3.1 µg	6%	24%	78%	5.13%	0.44%

DPPH ASSAY Root

Concentration (µg/mL)					
	Hexane	DCM	Chloroform	70% ethanol	water
50	62	75%	80%	62.70%	80.50%
25	59.5	74%	76%	59.80%	62.55%
12.5	24.5	52%	68%	25.30%	28.45%
6.2	18%	39%	65%	18.35%	9.55%
3.1	4%	29%	64%	3.70%	0.95%

DISCUSSION

Nothopodytes nimmoniana ion procedure with hexane, Di chloromethane, chloroform, aqueous ethanol(70% Ehanol) and water which showed the different yield extract in both root and leaf. Hexane showed 0.45%, Di chloromethane 3.9%, Chloroform 0.60%, aqueous ethanol 3.655 and water 0.90% yield of *Nothopodytes nimmoniana* Root extract with 67.5% of Insoluble residue with insoluble residue of 67.5% and total yield of 77% per 20 gms of Sample where as Nothopodytes nimmoniana Leaf consisted of Hexane 4.5%, Di chloromethane 12.015%, chloroform 1.04%, aqueous ethanol 10.8% and water 7.6% with insoluble residue of 45.5% and total of 81.5% of total yield per 20gm of sample. The extracts were further taken for phytochemical analysis. Starting with total carbohydrates of each extract, results show that chloroform root extract shows 0.42mg/ml and in leaf hexane and aqueous ethanol both 0.49mg/ml of total carbohydrate equivelance. Next, glucose oxidase and reducing sugars were done for the extracts which showed negligent amount of glucose and reducing sugars in the extracts. This showed the absence of sugars in this plant. Some phenolic groups are said to having cytotoxic activity because of its antioxidant property. Extracts of both root and leaf showed presence of phenol in it with leaf showing better result with 91.5% efficacy. Plants synthesize compounds with biological activity, namely antioxidant, as secondary products, which are mainly phenolic compounds serving in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage. Antioxidant assay such as FRAP and DPPH were done to analyse antioxidant activity present in the extract. Polyphenols could potentially be used as feed additive on in vivo trials and enhance the immune system of the receptors. The measurement of antioxidant activities, cannot be evaluated satisfactorily by a simple antioxidant test, without due regard to the many variables influencing the results. The correlation showed that DPPH assay is more accurate to measure the antioxidant activity of phenols with Chloroform being the most significant fraction 91% inhibition activity which can be taken for for furtur studies. The assay measures all compounds readily oxidizable under the reaction conditions and its very

inclusiveness allows certain substances to also react that are either not phenols or seldom thought of as phenol.

CONCLUSION

Results of the study conclusively affirm that the extract of *Nothopodytes nimmoniana* Root and Leaf showed significant results with Chloroform extract. To consider among root and leaf, Leaf is readily oxidable through antioxidant assay which can be taken further for cytotoxicity studies.

ACKNOWLEDGEMENT

I Would extend my sincere gratitude to the Research Schloras of Department of Dravyaguna JSS Ayurveda Medical college and Center of excellence and molecular biology and regenerative Medicine (CEMR) Lab, Department of Biochemistry for their kind support for carrying out my research work.

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