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Phyllanthus niruri: AS Antioxidant

[1*]Indu Thakur, [2]Pratima Sharma

[1*]Assistant Research Officer, Department of Research, Jawaharlal Nehru Cancer Hospital, and Research Centre, Idgah Hills, Bhopal462001.Madhya Pradesh, India.

[2] Assistant Professor, Institutes for Excellence in Higher Education 462001. Madhya Pradesh, India.

Abstract: **The aim of the present study was to evaluate extraction and the in-vitro free radical scavenging activity of Phyllanthus niruri. One or more of them phytoconstituents may be responsible for the antimutagenic, radioprotective properties. Qualitative phytochemical screening tests were performed to detect Phytochemicals in the different parts of herbs Aerial parts, Stem and Roots. Free radical scavenging activities of the different parts of herb extract were characterized by using Fenton reaction method. The result showed Antioxidant activity was found to be increased in a different concentration on the basis of dose dependent manner from 50-250 µg concentration with maximum Percent TBARS inhibition. The aerial parts have shown more active phytoconstituents and better free radical scavenging activity as compared to stem and roots of absolute alcoholic extract of Phyllanthus niruri.**

Keywords: Antioxidant, Fenton reaction, Phyllanthus niruri, Antimutagenic.

1. INTRODUCTION

Phyllanthus niruri (PN) is one of the herbs from the family of Euphorbiaceae and the extract from this plant was widely used in the preparation of various ayurvedic formulations [2].It is having very short life. P. niruri is a field weed and its genus Phyllanthus comprises of 600-700 species with minor distinguishing features among them. In Indian ayurvedic system extract is used as a medicine and is recommended for Bronchitis, Anaemia, Leprosy, Asthma, and Urinary disorders etc., In Charka Samhita. Herbal products have long been used in traditional folk medicine to maintain health or to provide remedies for various human diseases [1]. The analysis of the extract revealed several bioactive molecules and chemical agents including phyllanthin, hypophyllanthin, phyltetralin, niranthin, nirtetralin, hinokinin and isolintetralin [3, 4]. Which are lignans belonging the polyphenols group of compounds with well-known antioxidant properties [5]. Application of the extract exhibited antiulcer, antitumor and anticarcinogenic, hypolipidemic, antiviral due to its antioxidant properties, herbs has been prove for antimutagenic activity against various known mutagens.[6]In a preliminary study, it was observed that the aerial parts of the plant have radioprotective effect in mouse .[7]

2. MATERIALS AND METHODS

1. Identification, Collection and Preparation of *Phyllanthus niruri* extract-

Phyllanthus niruri is a rainy weed known as "Bhuiamalaki".Collection of plants has been done in the month of September– December 2016-2017. Herbs were collected from different Region like East zone with root 2452.17gm and without root 1343.05 gm, North zone got the herb with root 6198 gm without root 2198.05gm and cultivated in the campus of JNCH &RC 4 kg 1726 gm and South Zone contain with root 163.6 gm without root contain 77.95gm in the Bhopal area i.e., Bharat Havey Electrical Limited (BHEL area), Idgah hills, Govindpura, CIAE (Central Institute of Agricultural Engineering) Karond and different locality of Bhopal. Salamatpur and Vidisha also has been explore for herb collection, (Madhya Pradesh). The plant identified by principal Scientist at CSIR-National Botanical Research Institute (NBRI) Lucknow, and a voucher specimen was deposited in the Herbarium (voucher no. NBRI/D/2005/495).

Extraction process:

Three different types of extracts were prepared are:-

The absolute alcoholic extract of aerial part, stem and root were prepared by following process:-

Preparation of absolute alcoholic extract- 10 gm of coarse powder of aerial part, stem and root have been taken individually in three different beaker was refluxed with absolute methanol for 7 days 3hrs at 60^0C.

Phytochemical screening:

The color and consistency of the extract was noted and subjected to different tests to detect the presence of various phytoconstituents. The methods of Wagner's, Mayer's and Dragendroff's test for alkaloid (Peach and Tracey, 1955); Libermann- Burchard's, Salkowski and hemolysis test for steroid, triterpenoid and saponin (Finar, 1959); Benedicts and Fehling's test for reducing sugar (Wallis, 1985); lead acetate and vanillin-hydrochloride test for tannins and test for lignin (Kokate et al., 2001); Shinoda and alkaline reagent test for flavonoids (Shellard, 1957) was performed.

Isolation of active components with the help of Thin layer Chromatography (TLC):

10mg of concentrated extract was dissolved in 1ml of methanol, 10µl was applied on TLC plates made of silica gel GF_{254}(0.25 mm of thickness of gel). These plates were placed into the TLC chamber and allowed to run in the mobile phase of n-Hexane :Ethyl acetate (2:1) until it reaches a height of about 10 cm from the point of spotting. As the solvent moves upward sample travels by capillary action, it separates different active components on the plate appears like bands of different colors, which can also be visualized under UV light detector.

The R_f value of the bands was taken. Comparison of Rf values makes it possible to research complex mixtures qualitatively. The extent of the surface of the spot is a measure for the quantity of the material present (Fritz and Schenk, 1987).On the basis of R_f value, these bands were separated from silica gel and eluted by dissolving in absolute methanol individually in different vials.

Hydroxyl radical scavenging activity (Fenton Reaction):

The Fenton reaction was used to generate hydroxyl radicals in a test system, and the free radical scavenging activity was determined by the degradation of deoxyribose, as standardized by Elizabeth and Rao (1990). The hydroxyl radicals attack deoxyribose and initiate a series of reactions that eventually result in the formation of trichloroacetic acid reactive substance (TBARS). Radical scavenging by protectors results in inhibition of TBARS by protectors results in inhibition of TBARS. The reaction mixture consisted of deoxyribose (3 mM, 100 µl), $FeCl_3$ (0.1mM 100 µl), EDTA (0.1 mM 100 µl), H_2O_2 (1 mM 100 µl) and ascorbic acid (0.1 mM 100µl) in 550 µl of phosphate buffer saline (pH 7.4). Absolute alcoholic extract (50 µl) was added to the reaction mixture at concentrations of 50, 100, 150, 200,and 250µl to make a final volume of 1ml and incubated for 1 hr at room temperature. The mixture was then incubated for 20 min in a boiling water bath with 0.5 ml of 5 % Trichloroaceticacid and 0.5 ml of 1 % Thiobarbituric acid, cooled and centrifuged. The absorbance in the supernatant was measured at 532 nm in a UV-Spectrophotometer (Shimadzu, Japan). Dimethylsulfoxide (DMSO) was used as the positive control. The results are expressed as percentage inhibition of TBARS formation.

Pro-oxidant activity:

Several compounds, like some phenolic compounds which are antioxidants at lower concentrations. This can be tested by measuring the degradation of deoxyribose in the reaction mixture deprived of ascorbic acid (Paya *et al* 1992). The pro-oxidant activity of alcoholic extract Isolated components of Aerial part, stem and root of *Phyllanthus niruri* was tested by this method after adding 50 µl of 50-250 µl solution of alcoholic extract of each part to the reaction mixture devoid of ascorbic acid and measuring TBARS, as described above.

Ability to chelate –iron:

In the absence of EDTA, iron ions bind to the deoxyribose molecule and bring about site-specific damage in the molecule in the presence of ascorbic acid and H_2O_2 (Gutteridge 1984). The iron chelating property of aqueous and alcoholic extract of Aerial part, stem and root was tested in a reaction mixture devoid of EDTA to which was added 50µl of 50-250 µl solutions of both extract and measuring TBARS as above.

3. RESULT AND DISCUSSION

Phytochemical screening have been shown that the absolute alcoholic extract of aerial part isolation fraction S4 turned orange in colour but the colour was not stable it faded after few min. Fraction S2 and S3 from this detection method have almost the same Rf (0.81 and 0.89) and fraction S1 Rf (0.7) whereas fraction S4 Rf (0.9) by detection method .When alcoholic extract of stem was detected the fraction D2 and D3 shown the almost same Rf value (0.86 and 0.87) where fraction D1 shown (0.76). In case of P. niruri root isolated compound fraction was N1 and N2 Rf value was (0.85and 0.87).

The yield were calculated

Alcoholic extract Isolated compound - aerial part- 500ml (w/v) dark green, stem- 300ml (w\v) green, root- 360ml (w\v) light brown.

Aqueous alcoholic extract Isolated compound- aerial part- 600ml (w/v) dark brownish green, stem- 320ml (w\v) light brownish green, root- 370ml (w\v) light greenish brown.

Phytochemicals of *Phyllanthus niruri* in aerial part, stem, roots all alkaloids, flavonoids, Tannins, Steroids are rich in this herbs and therefore Rf value were compared with the reported Rf value by Vivian Fernand in May 2003.

Absolute alcoholic extract isolated compound of *P. niruri* inhibited the degradation of deoxyribose in-vitro in the Fe^{3+}–ascorbate–EDTA–H_2O_2 system, indicating hydroxyl radical scavenging ability. Isolated compound of aerial part showed significant hydroxyl radical scavenging activity in a dose dependent manner, whether the effect of stem isolated compound has been shown less inhibition whereas isolated compounds from root not shown good percent TBARS Inhibition. It appears that there is a saturation point in the dose response relationship of absolutealcoholic extract aerial part isolated compoundat the concentration of 200, and 250µl could be considered to be the optimum dose for antioxidant effect.

Alcoholic extract of aerial part isolated compound was also found to be devoid of pro-oxidant activity. In the absence of the chelator EDTA, the hydroxyl radicals generated by the Fe^{3+}–ascorbate–H_2O_2 system directly bind to deoxyribose and degrade it (Hofer, 2000). Reduction of Fe^{3+} to Fe^{2+} has been demonstrated to increase peroxidation and generate strong oxidants through auto-oxidation reactions of reduced iron (Satyamitra et al., 2001). Molecules which can inhibit deoxyribose degradation in the absence of EDTA are capable of complexing with iron, thus rendering it inactive. Absolute alcoholic extracts isolated compound were found to inhibit deoxyribose degradation in the absence of EDTA, suggesting inhibition of Fe^{3+} to Fe^{2+} conversion. The alcoholic extracts isolated compound showed good radical scavenging activity.

All the four compounds (S1, S2, S3, and S4) showed a dose-dependent increase in inhibition of TBARS.

S2 produced the least inhibition, 16.7% at 50µl/ml. S1 showed a higher effect with the maximum inhibition of 48 % at 250 µl /ml. S3 and S4 produced better inhibition, which was comparable to that produced by DMSO. S4 showed a slightly higher percent inhibition of 84% at 200 µl /ml even though it was not significant compared to that of S4 at 250 µl /ml.

The doses which has been produced maximum inhibitions (250µl/ml for S4 94%).

Epigenetic and alternative mechanisms of *Phyllanthus niruri* phytochemical in cancer treatment its components show free radicals scavenging activity and its different class of components with its mechanisms of action, like alkaloids work on upregulation of P53mand P73 with induction of apoptosis (Rana et al.,2010).Flavonoids, Flavon-3-ols effect on DNMT1 inhibition with cell invasion, cell proliferation and cell viability and Decrease CpG methylation with the activation of JNK pathways (Trainer 2012,Busch 2015,Zhao and H4 2013).

Isoflavones inhibit DNMT1, DNMT3 and DNMT3b with down regulation of hTERT expression and its regulate the miRNA expression. Whereas Lignans participate in cell cycle arrest at S-Phase as well as increase in cyclin-A expression (Ayella*et al.*,2010). Phenolic compounds also help in increase in Bax:Bcl2 ratio. Sakagomi et al., 2000 reported that Tannins present in *Phyllanthus niruri* work for the activation of caspases with the degradation of cytokeratin 18 which show DNA fragmentation. Those studies have been shown that all the active phytoconstituents are taken part in antioxidant, antimutagenic activity due to its free radical scavenging properties.

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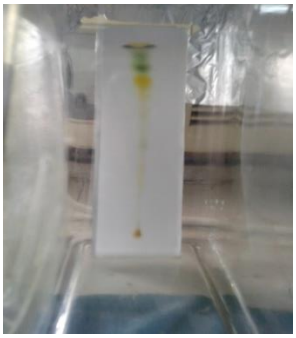
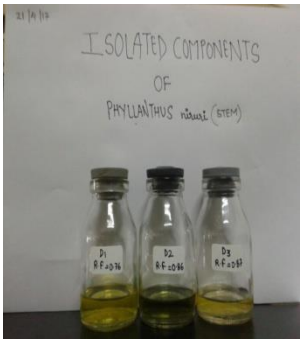
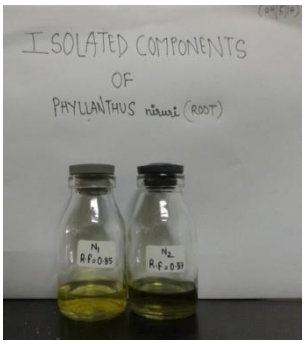


I.(a) Extraction of Aerial Part of *P. niruri* **(b) Isolation** **(c) Isolated Compounds**



II.(a) Extraction of Stem of *P. niruri* **(b) Isolation** **(c) Isolated compounds**



III. (a) Extraction of Root of *P. niruri* **(b) Isolation** **(c) Isolated Compounds**

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APPENDIX - A

I. Antioxidant activity of Phyllanthus niruri Aerial part, Stem and Root Isolated compounds:

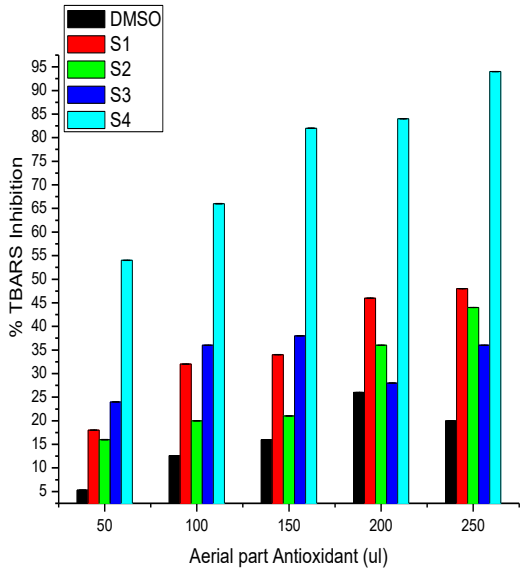

Fig 1 (a): In-vitro antioxidant activity from aerial part isolated compound of *Phyllanthusniruri*

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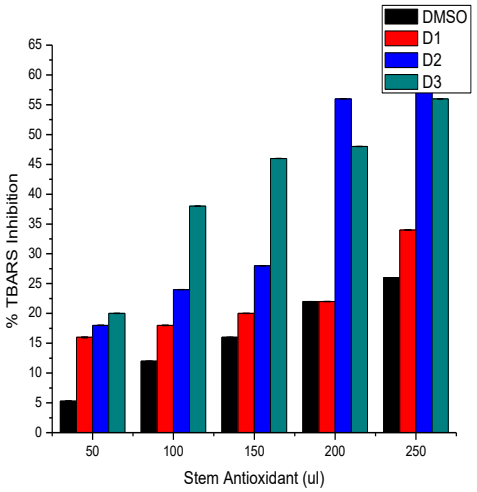

Fig 1 (b): In-vitro antioxidant activity from Stem part isolated compound of *Phyllanthus niruri*

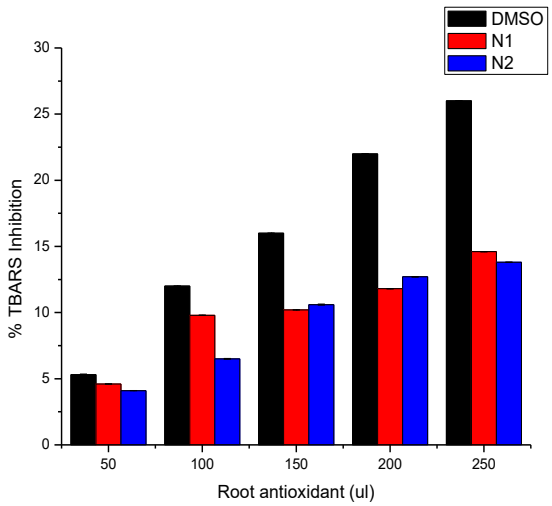

Fig 1(c): In-vitro antioxidant activity from Root part isolated compound of *Phyllanthus niruri*

II. Pro-oxidant activity of Aerial part, Stem and root of Isolated compounds:

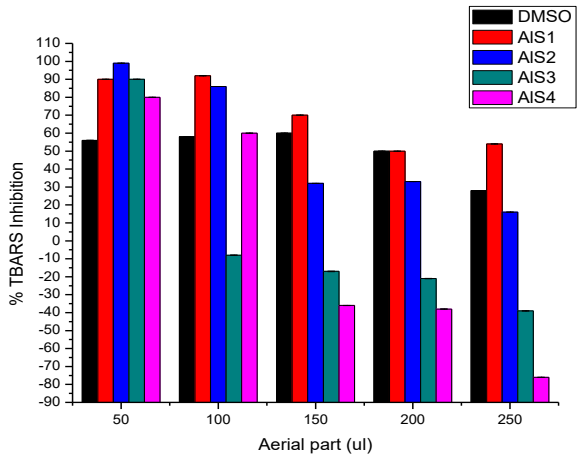

Fig II (a): In-vitro Pro-oxidant activity from Aerial part of isolated compound of *Phyllanthus niruri*

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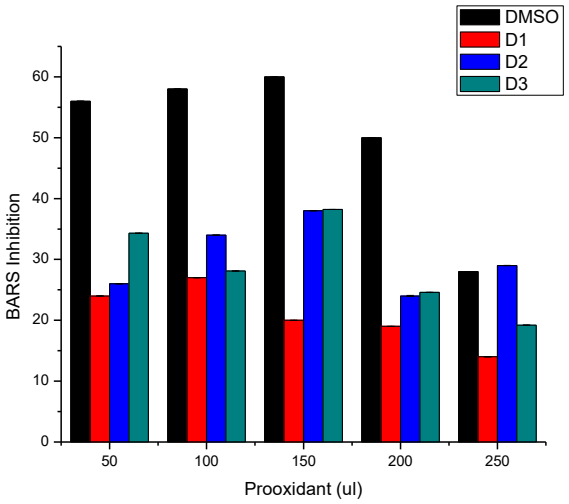

Fig II (b): In-vitro Pro-oxidant activity from Stem part isolated compound of *Phyllanthus niruri*

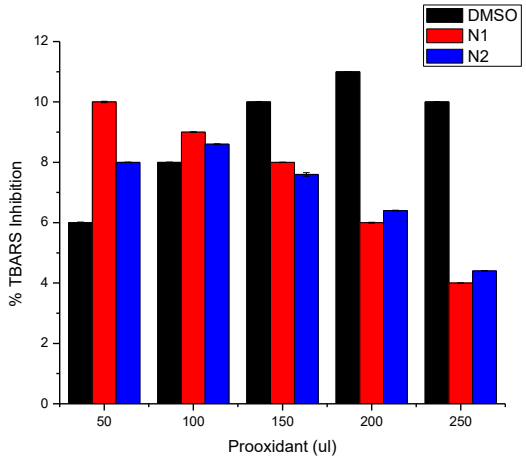

Fig II (c): In-vitro Pro-oxidant activity from Root part isolated compound of *Phyllanthus niruri.*

III. Chelate- Iron activity from Aerial part, Stem and root of Isolated compounds:

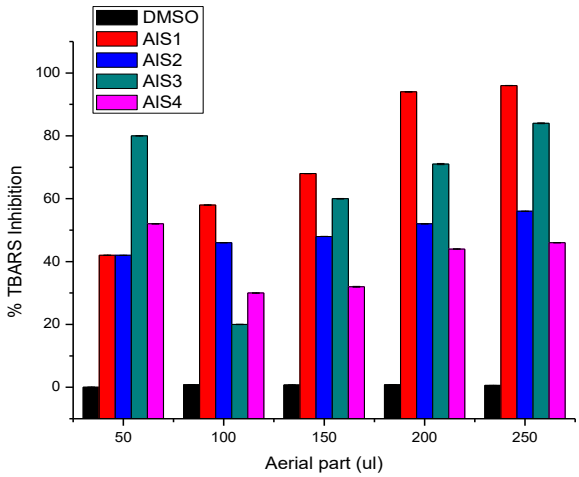

Fig III (a): In-vitro Chelate-Iron ability from Aerial part isolated compound of *Phyllanthus niruri*

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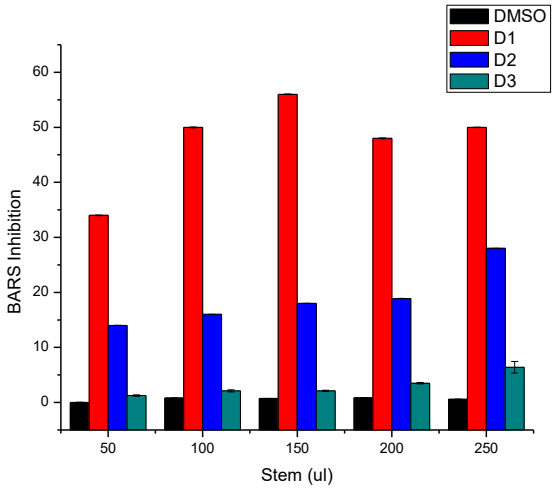

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Fig III (b): In-vitro Chelate-Iron ability from Stem part isolated compound of *Phyllanthus niruri*

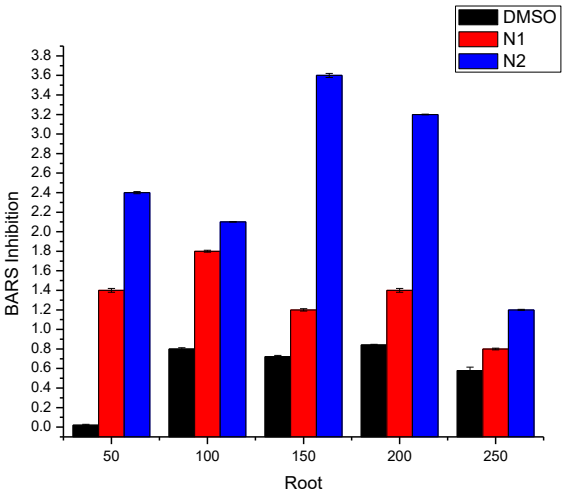

Fig III (c): In-vitro Chelate-Iron ability from Root part isolated compound of *Phyllanthus niruri*