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Comparative Evaluation of Crude Extract Fractions of the Whole Plant of *Taxillus heyneanus* and *Dalechampia indica* for Antioxidant Activity, Total Phenolic and Flavonoid Content

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Abstract: *This study was aimed to investigate the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant potential of ethanolic fraction (ETH&EDI) and chloroform fraction (CTH & CDI) of crude extracts of the whole plant of Taxillus heyneanus (TH) and Dalechampia indica (DI).To achieve this, several parameters such as scavenging activity (DPPH, Hydrogen peroxide and Nitric oxide), Reducing power assay, total flavonoids (TFC) and total phenolic content (TPC) using ascorbic acid and gallic acid as standard were examined. All the four extract fractions (ETH, CTH. EDI & CDI) exhibited antioxidant activity. However, the ethanolic fraction of T.heyneanus (ETH) presented a remarkable capacity to scavenge all the tested reactive species (DPPH, Hydrogen peroxide, Nitric oxide) with low IC_{50} values (38.75 ±0.83, 62.67±5.23, 80.89±0.47µg / ml). It also showed the highest TPC (125.40±5.24 mg GAE/g dry extract) and TFC (89.41 ±1.21 mg RE/g dry extract). The chloroform fraction of D.indica (CDI) showed low TPC (35.29±4.36 mg GAE/g dry extract), TFC (28.51±0.52 mg RE/g dry extract) and scavenging activity with high IC_{50} values(477.61±0.69, 404.00±0.38 & 505.52±4.83 µg /ml) values for the DPPH, Hydrogen peroxide and nitric oxide. Ethanolic fractions of both plants (ETH & EDI) exhibited significant scavenging activities with good amounts of phenolic and flavonoid compared to its chloroform fractions (CTH & CDI).The results of the study show that ethanolic fraction of Taxillus heyneanus possesses better antioxidant activity compared to the ethanolic fraction of Dalechampia indica which could be attributed to the presence of high amount of phenolic and flavonoids.*

Keywords: *Antioxidant activities, Dalechampia indica, Taxillus heyneanus, whole plant*

I. INTRODUCTOION

The human body is a wonderful machine which requires fuel named oxygen for its function. It plays a pivotal role in the energy metabolism of the body which involves various physical and chemical processes [1] .While oxygen supports our life, it also produces free radicals (ROS) in the body as the waste products. Oxygen-centered free radicals (ROS) includes superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (.OH), hydrogen peroxide (H_2O_2), peroxyl, hypochlorous acid and nitric oxide [2]. These are highly reactive unstable molecules which have a hunger for electrons, reacts with biomolecules (lipids, proteins, DNA etc.) and alters its structure and function. This, in high levels, leads to oxidative stress [3, 4].Oxidative stress occurs when our body defense systems fail to wipe off this excess amount of free radicals. These free radicals spring from different sources such as endogenous (mitochondria, peroxisomes, endoplasmic reticulum etc.) and exogenous (pollution, ionization radiation etc.) sources [5].It has implications for a broad range of disorders including stroke, ischemia-reperfusion injury, arthritis, cancer, diabetes, neurodegenerative diseases, spinal cord injury, cardiovascular diseases, sepsis, inflammation and respiratory diseases [6, 7].Here comes the concept of antioxidants, nature's way of cell defense against free radical (ROS) attack. These antioxidants synthesized *invivo (*SOD, catalase, *glutathione peroxidase)* or taken as dietary antioxidants (vitamin C, vitamin E, β-carotene and selenium) either quenches or scavenges free radicals (ROS) and repairs the damages caused by them [8].The quest for effective and beneficial natural antioxidants has been increased nowadays as the synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) found to be harmful [9].Plants are found to be a good source of natural antioxidants as they can synthesize phytoconstituents such as phenolics, tannins, flavonoids etc. in different parts which have the ability to scavenge free radicals [10].

Taxillus heyneanus Danser (TH) and *Dalechampia indica* Wight (DI) plants were selected for the study. *T. heyneanus* (Loranthaceae), commonly called as badanika is a parasitic herb on shrubs and small trees common in dry deciduous forests of Andhra Pradesh, Karnataka, Kerala and Tamilnadu. Branches are densely covered with white shiny abietiform hairs intermixed with stellate hairs. Branchlets are tawny pubescent Nodes are prominently ribbed with leaf scars. Leaves are alternate, orbicular to ovate, attenuate at base, rounded at apex, crustaceous and evanescently hairy on both surfaces. The petiole is hairy to glabrous. Axillary Inflorescence which is shortly peduncled or sessile simple dichasium. Flowers are 5-merous with long pedicels. Bracts are obovate to oblanceolate and foliaceous. Calyculus is five lobed. Petals (5) are united to form a

ventricose tube with a median split. Stamens are 5 in number. Oblong anther, turbinate ovary and bifid or depressed stigma are present. Fruits are broadly ellipsoid, crowned by persistent calyculus [11-13].

Dalechampia indica (Euphorbiaceae), commonly called as aliparnika, is a twining undershrub commonly found in dry hills of Andhra Pradesh and Tamil Nadu. This is finely pubescent with stinging hairs except on upper surface of leaves. Leaves are foliolate, chartaceous, glabrous whereas leaflets are sessile or shortly petiolulate, which is acute to rounded at base and short-acuminate at the apex. Flowers are surrounded by fleshy scales formed out of deformed flowers. Female flowers are ciliate with stalked glands along margins; pubescent globose ovary, long style, copular stigma, depressed-subglobose fruits, glandular hairy fruiting calyx and mottled dark grey globose seeds are present [14.15].

Though, both the plants come under the category of medicinal plants, the exact traditional uses of them were not mentioned in the literature. In the present study, the whole plant of *Dalechampia indica* (DI) and *Taxillus heyneanus* (TH) were evaluated for DPPH, Hydrogen peroxide, Nitric oxide radical scavenging activities, Reducing power assay, Total phenolic content (TPC) and Total flavonoid content (TFC).

II. MATERIALS AND METHODS

2.1 Plant material Collection and Authentication

The whole plant of both *T. heyneus* and *D. indica* were collected in the month of April, 2014 from chittur dist. The plant materials were identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference.

2.2 Chemicals

The chemical agents such as 1,1-Diphenyl-2-picrylhydrazyl (DPPH) , ferric chloride ($FeCl_3$), Folin-Ciocalteu Phenolic reagent, Sodium bicarbonate, Potassium ferricyanide, L- Ascorbic acid, ethanol, chloroform, PET ether, Ethylenediamine tetraacetic acid (EDTA),Ferric chloride, sodium nitroprusside, Hydrogen peroxide (H_2O_2), rutin, glacial acetic acid, sulphanilamide, Trichloroacetic acid (TCA) and Sodium hydroxide were purchased from Sigma Chemicals Co. (St. Louis, USA) or Himedia, Mumbai. All the chemicals used were of analytical grade.

2.3 Preparation and Fractionation of crude extract of plant material

Plant materials were first chopped, then washed with fresh water to remove dirt and other contaminants. They were shade-dried for several days with occasional sun drying. The dried materials were pulverized into coarse powder by a grinding machine and the materials were stored at room temperature (RT) for future use. The dried coarse powder (150g) of plant extracts was macerated with absolute ethanol at room temperature for a period of 10 days with frequent agitation in clean, sterilized and properly sealed flat bottomed glass container. The solution was then subjected to filtration on a piece of clean, plain sterilized cotton cloth. The resulting filtrate was concentrated on a water bath maintaining 40°C to dryness. The concentrate obtained was designated as crude extract [16]. The crude extracts (10g) of each plant were subjected to fractionation by suspending in EOH: H_2O (3:7; v/v) and extracted successively with petroleum ether and chloroform in this order using separating funnel. Dried plant extracts of different solvents were weighed and stored for future use [18]. PET ether fraction of the plants formed was very little which is insufficient to carry out analysis. In the present study, except PET ether extracts, the ethanolic (ETH & EDI) and chloroform fractions (CTH & CDI) of crude extracts of *T. heyneus* and *D. indica* were analyzed for *invitro* antioxidant activity, total phenolic and total flavonoid content.

2.4 Estimation of Total Phenolic Content (TPC)

The phenolic content in plant extracts was determined using spectrophotometric method [17]. The reaction mixture was prepared by mixing 0.5 ml (1 mg/ml) sample solution of extract with 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml ethanol, 2.5 ml 10% Folin-Ciocalteu's reagent and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45^oC for 45 min. The absorbance was measured using spectrophotometer at 765 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid (25, 50, 75,100 & 250 µg/ml) and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics in the extract was read (mg/ml) from equation obtained from the constructed calibration line of gallic acid and expressed in terms of gallic acid equivalent (mg of GAE/g of extract) [19].

2.5 Total Flavonoid Content estimation (TFC)

Total flavonoid content of different fractions of plant extracts (ETH, EDI, CTH & CDI) was measured by the aluminum chloride colorimetric assay.1 ml of sample extract solution of concentration 1mg/ml and 4 ml

of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was added and allowed to stand for 5 minutes, then 2 ml of 1M Sodium hydroxide was treated and volume was made up to 10 ml with distilled water. A set of reference standard solutions of rutin (25, 50, 75, 100 and 250 µg/ml) were prepared in the same manner as described earlier. The absorbance of test and standard solutions were determined against the blank at 510 nm using UV/Visible spectrophotometer [20]. Based on the measured absorbance, the concentration of flavonoids in the extract was read (mg/ml) from equation obtained from the Rutin calibration line and was expressed was expressed as mg of RE/g of extract.

2.6 DPPH free radical scavenging assay

The free-radical scavenging activity of the plant extracts was determined by the method described by Chang et al using DPPH with some modifications [21]. Various concentrations (25, 50, 75,100 & 250µg/ml) of plant extracts were obtained from stock solution (1 mg/ml) by serial dilutions in respective solvent systems. In this method, 1 ml of extract solutions of various concentrations was added to freshly prepared 4ml of 0.004% of the ethanolic DPPH solution and mixed properly. Then the solutions were allowed to stand for 30 mins at room temperature in dark for the chemical reaction to occur. After half an hour, absorbance was measured against blank at 517 nm using a UV-Visible spectrophotometer. Ethanol served as blank. Ascorbic acid used as a standard. A control sample was prepared in a similar way without adding any extract and standard solution in the reaction mixture. The percentage scavenging activity of plant extracts was calculated as:

$$\% \text{ scavenging activity} = (Ab_{control} - Ab_{sample}/Ab_{control}) \times 100 \quad (1)$$

Where $Ab_{control}$ indicates absorbance of control & Ab_{sample} indicates absorbance of sample

2.7 Reducing power assay

Oyaizu method was followed to determine the reducing power of different fractions (ETH, EDI, CTH and CDI) of crude extracts [22]. One ml of extract solution of various concentrations (25, 50, 75,100,250 µg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of (1% w/v) potassium ferricyanide [K3Fe(CN)6]. This mixture was incubated at 50 °C for 20 min. By adding 2.5 ml of 10% trichloroacetic acid the reaction was terminated and centrifuged at 3000 rpm for 10 min. After centrifugation, 2.5 ml of the supernatant solution was mixed with distilled water (2.5 ml) and 0.1% ferric chloride (0.5 ml) solution. Then the absorbance was read at 700 nm against a blank using UV spectrophotometer. Increased absorbance value of the reaction mixture indicates increased reducing power. Each test sample was made in triplicates and average data was noted. Here, ascorbic acid was standard.

2.8 Hydrogen peroxide scavenging assay

Scavenging activity of plant extracts and its subfractions (ETH, EDI, CTH and CDI) were determined by Ruch et al method with slight modification using hydrogen peroxide [23]. 40 mM of the H_2O_2 solution was prepared in PBS (pH 7.4). 4 ml of plant extract solution prepared in ethanol at various concentrations (25, 50, 75,100,250 µg/ml) was mixed with 0.6 mL of 40mM H_2O_2 solution and incubated for 10 min. The absorbance of the solution was determined at 230 nm against a blank containing the extract in PBS without H_2O_2. Ascorbic acid was used as a standard. The amount of hydrogen peroxide radical scavenged by the extract was calculated using the following equation:

$$\% \text{ Inhibition} = (A_{control} - A_{test}/A_{control}) \times 100 \quad (2)$$

Where $A_{control}$ indicates absorbance of control & A_{sample} indicates absorbance of sample

2.9 Nitric oxide scavenging assay

This assay was determined by Griess Illosvoy reaction using sodium nitroprusside [24]. 0.5 ml of extract fractions (ETH, EDI, CTH and CDI) at various concentrations (25, 50, 75,100,250 µg/ml) was mixed with 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) and incubated at 25°C for 150 minutes. From this mixture, 0.5 ml was taken out and 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) was added to it. It was further incubated at room temperature for 5 more minutes. Finally, 1.0 ml (0.1% w/v) naphthyl ethylenediamine dihydrochloride was mixed and allowed to stand at room temperature for 30 minutes. A control solution contained the same solution mixture without plant extract or standard. Ascorbic acid was used as a standard. The absorbance of the control, standard and sample solution was measured at 546 nm. The percentage inhibition was calculated according to the following equation:

$$\text{NO Scavenged (\%)} = (A_{cont} - A_{test})/A_{cont} \times 100 \quad (3)$$

Where A_{cont} is the absorbance of the control and A_{test} is the absorbance of the extract solution

2.10 Statistical Analysis

All the experiments were done in triplicates. The experimental results are expressed as mean ± SD of triplets. Statistical analysis was performed using Graph Pad Prism Software, Version 7 (Graph Pad Software, San Diego, CA, USA).

III. RESULTS AND DISCUSSION

Most of the natural antioxidants perform different functions. Therefore, a reliable antioxidant evaluation protocol requires different assessments of antioxidant activity to account various mechanisms of action. In this study, several techniques have been used to determine the *invitro* antioxidant activity to allow rapid screening of substances such as DPPH, Hydrogen peroxide, Nitric oxide scavenging activity and reducing power assay. The fractions were also evaluated for the presence of phenolic and flavonoid content which are responsible for antioxidant activity.

3.1 Total phenolic content

Phenolic content in the given plant extracts was determined by using Folin-Ciocalteu reagent. This is also called as Gallic acid equivalence method as the total phenolic amount was calculated from the equation ($y=0.0084x+0.3499$, $R^2 = 0.991$) obtained from gallic acid standard curve (Fig 1A) and results were expressed as mg gallic acid equivalents (GAE)/g dry extract. This method is preferred more because of its accuracy. In the present study, the ethanolic extracts (ETH & EDI) of the plants *T. heyneanus* and *D. indica* showed comparatively high phenolic content (125.40±5.24,71.54±3.58 mg GAE/G dry extract) than chloroform (CTH & CDI) extracts (67.42±1.37,35.29±4.36 mg GAE/G dry extract) respectively. Among all ETH showed high phenolic content whereas CDB showed the least. The results were given in Table 1 and Fig 1C.

Phenolic compounds are the plant's secondary metabolites with one or more hydroxyl groups linked to aromatic rings, perform various physiological functions which include antioxidant activity as they are excellent oxygen radical scavengers and free radical terminators. Several studies have shown the close relationship between the total phenolic amount and antioxidant activity [25].

3.2 Total flavonoid content

Flavonoid content in plant extracts was obtained by Aluminum chloride colorimetric method using $Alcl_3$.It involves complex formation between the aluminum ion, Al (III), and the carbonyl and hydroxyl groups of flavonoids to produce characteristic yellow color [26]. Total flavonoid content was calculated from equation ($y=0.009x+0.157$, $R^2 = 0.996$) obtained from the standard calibration curve of rutin (Fig 1B). The ethanolic fraction of *T.heyneanus* extract showed highest flavonoid content (89.41±1.21mg RE/g dry extract) against other fractions (CTH, EDI, CDI) whose flavonoid content reported as 52.27±2.44,64.51±1.94 & 28.51±0.52 mg RE/g of dried weight, respectively. The results were seen in Table 1 & Fig 1D.

Flavonoids are polyphenolic plant metabolites that perform different functions and provide various potential health benefits to humans because of their antioxidant nature, anti-inflammatory, anticancer, antiviral, antibacterial, vasodilatory and immune stimulating activity[27].Flavonoids mediate their antioxidant effects by scavenging free radicals or by chelating metal ions or by inhibiting generation of free radicals [28].

3.3 DPPH free radical scavenging activity

Scavenging activity of crude extract fractions was determined based on their DPPH neutralization. The percentage scavenging activity of various concentrations (25, 50, 75,100,250 µg/ml) of all extract fractions and ascorbic acid (5, 10, 15, 20, 25µg/ml) determined, was found to be concentration dependent. More the scavenging activity, less is the IC_{50} value. ETH showed maximum free radical scavenging activity with IC_{50} 38.75±0.83 µg/ml when compared to other fractions (EDI, CTH, CDI) whose IC_{50} Values were 135.04±3.99, 105.72±5.11, 477.61±0.69 µg/ml against the standard (7.56±0.24µg/ml). *T.heyneanus* fractions (ETH &CTH) showed good scavenging ability than *D.indica* fractions (EDI & CDI). Fig 2A, Table 2, and Fig 3 represents the percentage of DPPH neutralization activity and IC_{50} of all extracts considered in the study.

DPPH (2, 2-diphenyl-1-picryl-hydrazyl) is a stable, free radical that produces purple color in alcohol. This color fades in presence of antioxidant molecule as it converts into 2, 2-diphenyl-1-picryl-hydrazine.More the scavenging ability less the absorbance detected at 517nm.This method was chosen as it is easy to perform and gives accurate results [29].

3.4 Reducing power assay

All four extracts fractions (ETH, CTH, EDB, and CDB) showed concentration-dependent reducing activity with increasing absorbance. Among all, ETH showed high reducing activity with more absorbance (0.883±0.03) when compared to other extracts (CTH, EDI, CDI) absorbance 0.365±0.04, 0.676±0.05, and 0.122±0.03 respectively at 250µg/ml concentration. The absorbance of ascorbic acid at its high concentration

was 1.268±0.06. Fig 2B describes the reducing power of four extract fractions and ascorbic acid.

In reducing power assay, the test solution changes color from yellow to green depending on the reducing power of the test sample. Antioxidants cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form which can be determined by absorbance measurement at 700 nm. Increasing absorbance at 700 nm indicates an increase in reducing ability [30]. The antioxidants present in the ethanolic fractions of *T. heyneanus & D.indica* caused the reduction of Fe^{3+} / ferricyanide complex to the ferrous form and thus proved to have good reducing power [31].

3.5 Nitric oxide scavenging activity

Figure 3 and Table 2 represents the comparative IC_{50} values of various fractions of plant extracts and ascorbic acid (standard) were shown. All extracts have shown a significant amount of carrying activity which indicates antioxidant effect (Fig 2D). ETH has shown low IC_{50} (80.89±0.47µg/ml) whereas CDI showed high IC_{50} (505.52±4.83 µg/ml).Ascorbic acid showed 18.47±1.24 µg/ml IC_{50} value. Fig 2C shows the nitric oxide scavenging activity of extract fractions and ascorbic acid.

Nitric oxide (NO), an important chemical mediator generated by the human body responsible for altering structural and functional components of cell associated with several diseases [32]. In this assay, nitric oxide generated from sodium nitroprusside interacts with oxygen to produce nitric ions which can be estimated by the use of Griess reagent at 546 nm. Scavenger of nitric oxide competes with oxygen, leading to reduced production of nitric oxide indicated by a decrease in the absorbance [33]. This decrease in absorbance of ETH is attributed to the presence of phenolics and flavonoids that scavenge free radicals.

3.6 Hydrogen peroxide scavenging activity

50% Hydrogen peroxide scavenging activity (IC_{50}) of different extract fractions is presented in Fig 3 & Table 2. High IC_{50} values of CTH, EDB, and CDB (128.80±1.49, 153.80±9.61, 404.00±0.38 µg/ml) indicate less antioxidant activity than ETH (62.67±5.23 µg/ml).The IC_{50} value of standard was found to be 22.65±0.51 µg/ml. Percentage scavenging of hydrogen peroxide of all extract fractions and standard were given in Fig 2C.

Hydrogen peroxide, cytotoxic agent contribute to oxidative stress indirectly by generating highly reactive toxic hydroxyl radicals in the body which show deleterious effects on the cells by readily reacting with biological molecules [34]. The ethanolic fraction of *T. heyneanus* efficiently scavenged H_2O_2 may be attributed to the phenolic compounds that could donate electrons to hydrogen peroxide, thereby neutralizing it into water.

Table1: Total phenolic and Total flavonoid content of the plant extract fractions

Sample	Extract	Total Phenolic Content (TPC) mg of GAE/g of dry extract	Total flavanoid content (TFC) mg of RE/g of dry extract
Taxillus heyneanus	Ethanolic (ETH)	125.40±5.24	89.41±1.21
	Chloroform (CTH)	67.42±1.37	52.27±2.44
Dalechampia.indica	Ethanolic (EDI)	71.54±3.58	64.51±1.94
	Chloroform (CDI)	35.29±4.36	28.51±0.52

Values presented as the mean ± SD, *n* = 3

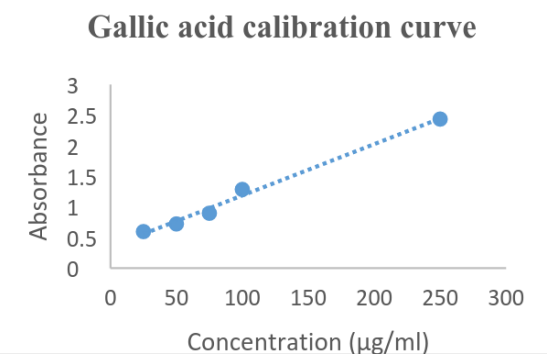
Table2: IC_{50} values of different extract fractions of plants and Ascorbic acid for various scavenging activities

Sample	Extract	IC_{50} (µg/ml) DPPH	HSA	NSA
Taxillus heyneanus	Ethanolic (ETH)	38.75±0.83	62.67±5.23	80.89±0.47
	Chloroform (CTH)	105.72±5.11	128.80±1.49	176.00±5.60
Dalechampia.indica	Ethanolic (EDI)	135.04±3.99	153.80±9.61	100.41±0.96
	Chloroform (CDI)	477.61±0.69	404.00±0.38	505.52±4.83
Ascorbic acid	----------	7.56±0.24	22.65±0.51	18.47±1.24

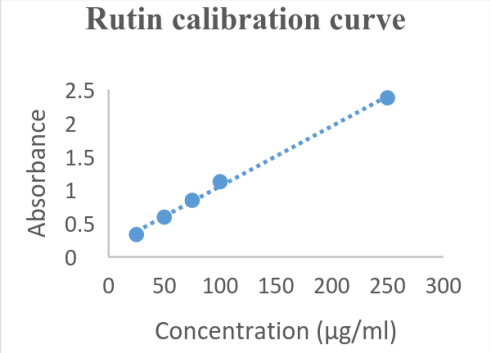
Values presented as the mean ± SD, *n* = 3

Fig 1: Total Phenolic and Flavonoid content of various extract fractions of plants *T.heyneanus* & *D.indica*

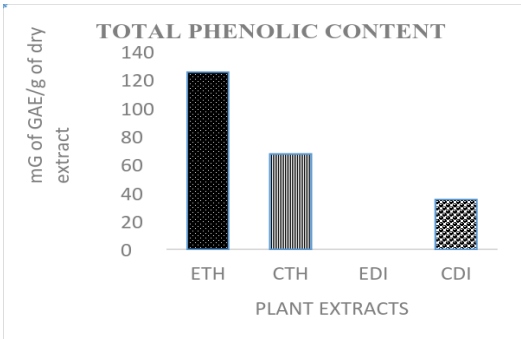
A. Calibration curve of Gallic acid



B. Calibration curve of Rutin



C. Total Phenolic Content of plant Extract Fractions Fractions



D. Total Flavonoid Content of plant Extract

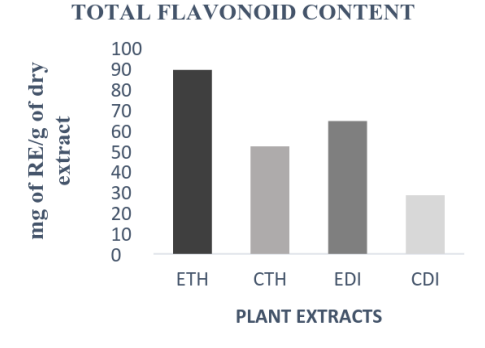
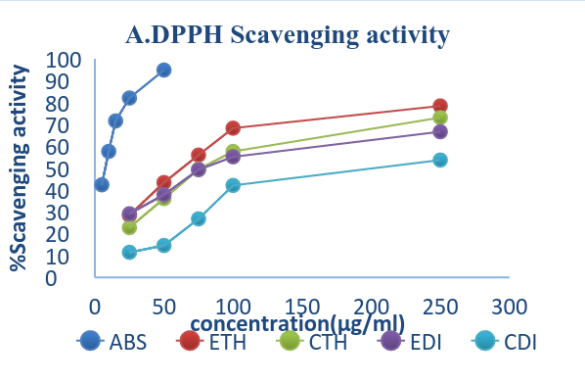
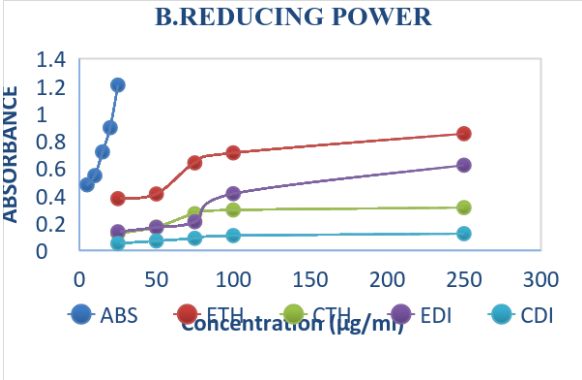


Fig 2: Various Scavenging Activities of Different Extract Fractions of *T.heyneanus, D.indica* and Ascorbic Acid





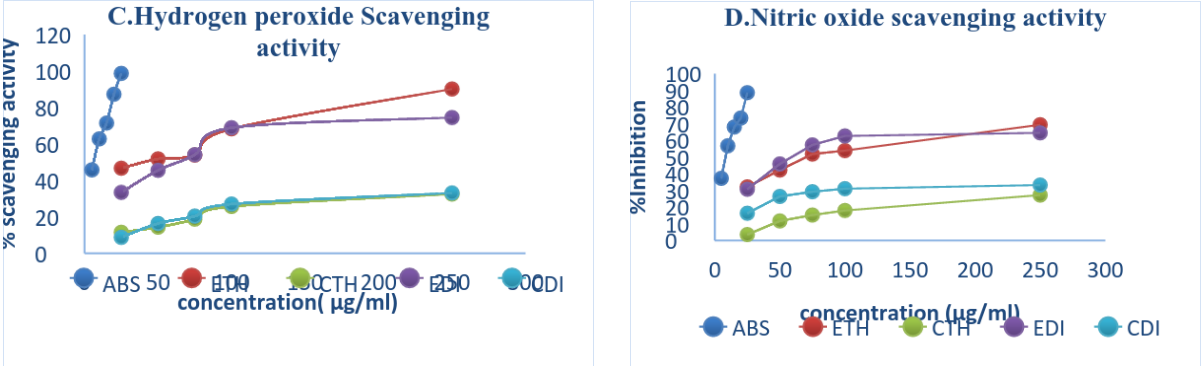
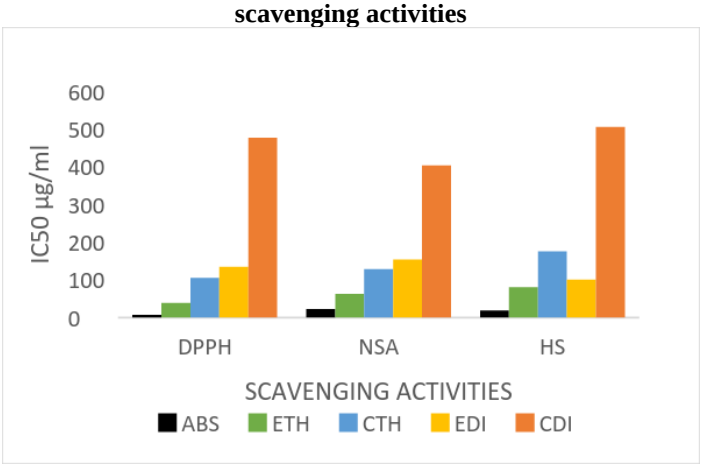


Fig 3: Comparative IC_{50} values of Ascorbic acid, *T.heyneanus* and *D.indica* extracts fractions for various scavenging activities



IV. CONCLUSION

Both ethanolic and chloroform fractions of the plants *T. heyneanus & D.indica* were evaluated. The results clearly indicated that *T.heyneanus* fractions (ETH & CTH) tested in this investigation possess significant antioxidant activity than *D.indica* fractions (EDI&CDI).ETH exhibited high phenolics, flavonoid contents and also, high antioxidant activity with a low IC_{50} suggests that phenolic compounds, including flavonoids, are the main contributors of antioxidant activity in these species. However, to the best of our knowledge, this is the first report of investigation on the antioxidant capacity and total phenolics as well as flavonoid content of *T. heyneanus & D.indica* species. However, further detailed investigation, especially *invivo* antioxidant and toxicity studies are needed to justify its use as a natural source of antioxidants to prevent the progression of many diseases.

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Abbreviations

ETH: Crude ethanol extract of *T.heyneanus*; CTH: Chloroform fraction of *T.heyneanus*; EDI: Crude ethanol extract of *D.indica*; CDI: Chloroform fraction of *D.indica*; ABS: Ascorbic acids; GAE: Gallic acid equivalent; RE: Rutin equivalent; SD: Standard deviation; TPC: Total phenolic content; TFC: Total flavonoid content.

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