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Comparative phytochemical and antioxidant activity of *Trewia nudiflora* leaf and fruit

Mohammad Mostafa Mohsin Milon, Ranjan Kumar Sarker, Razia Sultana and GM Masud Parvez

Abstract
The main intention of the study was comparative phytochemical and antioxidant activity screening of leaf, peel and flesh parts of the medicinal plant *Trewia nudiflora* in terms of their phenolic content, flavonoid content, total antioxidant capacity, reducing power capacity and radical scavenging capacity. The leaf, peel and flesh were dried and grinded to course powder and extracted with methanol and ethyl acetate solvents. Among methanol and ethyl acetate extracts, methanolic extract of leaf of *Trewia nudiflora* (LTNM) carried the maximal amount of phenolic content 14.537±0.696 mg of GAEs/gm and ethyl acetate extract of flesh of *T. nudiflora* (FTNE) comprised the most amount of phenolic content 14.298±0.680 mg of GAEs/gm. In our study, among all the extracts, methanolic extract of leaf of *Trewia nudiflora* (LTNM) possessed maximum amount of flavonoid content 5.398±0.414 mg of CAEs/gm. It was observed that the leaf of *Trewia nudiflora* (LTNM) contained the highest amount of antioxidant capacity 29.311±0.429 mg of AAE/g. Among all the extracts, leaf of *Trewia nudiflora* (LTNM) signified the greatest reducing power capacity. Among all the methanolic extract the leaf (LTNM) showed the utmost scavenging activity 72.517% that was the closest to the standard BHT 83.427%. The flesh (FTNE) showed the most scavenging activity (70.909%) among all ethyl acetate extracts. In our study, the IC_{50} value ranged from 40.95 to 172 µg/ml except the ethyl acetate extract of leaf. This indicates significant radical scavenging activity. Finally, the leaf extract had given the most significant phytochemical and antioxidant activity in all the cases.

Keywords: Phytochemicals, antioxidant, phenolic content, IC_{50}, DPPH, extracts, free radical

Introduction
Medicinal plants provide complementary treatments with great potential. In addition to giving poor people access to affordable medical care, they can also help developing nations by creating jobs, money, and foreign exchange. Agreeing to WHO, more than 80% of the world's population depends basically or exclusively on conventional medicine for their medical needs [1]. Typically, medicinal plants are defined as those that have beneficial pharmacological effects on humans or have therapeutic qualities. Flavonoids, saponins, resins, lactones, alkaloids, sterols, glycosides, tannins, quinines, and other secondary metabolites, among others, are naturally produced and accumulated by medicinal flora [2].

Free radical reactions are recognized to have a great component inside the pathophysiology of many acute and chronic human diseases, including diabetes, atherosclerosis, aging, immunosuppression, and neurodegeneration [3]. The antioxidant properties of medicinal plants may help explain why they provide disease protection. Morbidity and mortality from degenerative diseases have been shown to be negatively correlated with dietary intake of natural antioxidants [4].

Trewia nudiflora L. (Euphorbiaceae), a tropical plant basically conveyed in India and its neighboring nations, Malaysia and south China, relishs a massive reputation in conventional herbal medication [5]. Numerous parts of *T. nuliflora* such as entire plant, fruit, leaves, and seeds have been specified for pharmacological activities [6]. It holds pyridine alkaloid, N-methyl-5-carboxamide-2-pyridone, nudiflorine and Bark yields taraxerone and betasitosterol. Seeds have ricinidine alkaloid and also include a maytansinoid compound, trewiasine [7]. The ethanolic and aqueous extracts of roots and leaves of *Trewia nudiflora* offered the large antioxidant activity [8]. So, the main motive of the work was the comparative phytochemical and antioxidant activity screening of leaf, peel and flesh part of the medicinal plant *Trewia nudiflora*.

Methodology

Collection and Preparation of *T. nudiflora* Leaves, Peel and Flesh

The leaves and fruits of the tree were collected from Pabna, Rajshahi area of Bangladesh. To begin, fruits and leaves thoroughly washed with water and shed dried. After that, the leaf, peel and flesh were oven dried at 50° C and the plant parts were grinded to course powder by using a blender. At last, powder stored in a cool, dim and dry spot and protected in hermetically sealed holders for further utilization.

Extraction

50 g of the leaf, peel and flesh were placed in plastic cap containing glass bottle and conical flask. Then, the solvent methanol and ethyl acetate where added separately in each of the sample containing container (200 ml of solvent for each 50 g powder). After the addition of solvent the samples were extracted using a thermostat incubator with shaker machine (IN-SK 100, Wincom Company Ltd., China). The shaking process continued for 2 days (5 hours each day giving 15 minute interval after each hour) at 37°C temperature. To get the maximum extract, the sample was extracted by two times. After that, preparation was filtered using markin cloth followed by filtration with filter paper and filtrate was collected separately in beakers. Then, the filtrate was evaporated at room temperature until the sticky concentrated mass of methanolic extract of leaves of *T. nudiflora* (LTNM), ethyl acetate extract of leaves (LTNE), methanolic extract of peels (PTNM), ethyl acetate extract of peels (PTNE), methanolic extract of flesh (FTNM) and ethyl acetate extract of flesh (FTNE) were obtained.

Determination of Total Phenolic Content

According to (Singleton *et al*., 1965; Ainsworth EA & Gillespie KM, 2007; Alothman M *et al*., 2009) [9-11], gallic acid (GA) was employed as the standard and the Folin-Ciocalteu reagent (FCR) as the oxidizing agent to evaluate the total phenolic content of various extractives. In the test tubes, 0.4 ml of extractives or standards in various concentrations were first added. Then 2.0 ml of the FCR reagent solution (diluted 10 times with water) was put into each test tube. Then, 2.0 ml of a sodium carbonate solution (7.5%) was added to each test tube. The reaction was then completed by incubating the test tubes for 20 minutes at 25°C. Next, the solution's absorbance was assessed at 760 nm using a spectrophotometer against blank. The following formula was used to determine the amount of total phenolic compounds in methanol extract and various fractionates expressed in gallic acid equivalents (GAE):

$$C = (c \times V)/m$$

Where, C is the amount of total phenolic compounds in mg/g of plant extract in GAE, c is the concentration of GA in mg/ml determined by the calibration curve, V is the extract volume in ml, and m is the weight in gm of the various plant extracts.

Determination of Total Flavonoid Content

The aluminum chloride colorimetric method was used to assess the total flavonoid content of various extractives. The extractives' total flavonoids concentration was reported as mg of CAE/gm of dry extract using CA (catechin) as the standard (Kiranmai M *et al*., 2011; Akbay P *et al*., 2003; Sultana B *et al*., 2008) [12-14]. First, 0.5 ml of a standard or extractive solution at various concentrations was added to the test tubes. Then each of the test tubes received 1.5 ml of methanol. Each test tube then received 100 µl of the 10% aluminium chloride solution. After that, 100 µl of 1M potassium acetate solution was added to each test tube. The test tubes had distilled water (2.8 ml) added to them. The test tubes were then incubated at RT for 30 minutes to complete the reaction. The solution's absorbance was measured using a spectrophotometer at 420 nm. The following formula was used to calculate the total compounds in the extracts.

$$C = (c \times V)/m$$

Where, C = total content of flavonoid compounds, mg/g of plant extract in GAE, c = the concentration of CA in mg/ml established from the calibration curve, V = the volume of extract in ml, m = the weight of plant extracts in gm.

Determination of Total Antioxidant Assay

With slight changes, the total antioxidant capacity of various extractives was calculated in accordance with (Prieto *et al*., 1999; Mashwani Z *et al*., 2013) [15-16]. First, test tubes were filled with 0.5 ml solutions of various extractives or standards at various concentrations. Then, each test tube received 3 ml of reaction solutions containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 1% ammonium molybdate. The reaction was then completed by incubating the test tubes at 95 °C for 10 minutes. After cooling at RT, the solutions' absorbance at 695 nm was measured using a spectrophotometer against a blank. Typically, 3 ml of reaction mixtures and the required volume (300 µl) of the same solvent were employed to create a blank solution. A typical blank solution was made up of 3 ml of reaction mixtures, 300 µl of the same solvent as the sample, and was incubated under the same conditions as the rest of the sample solution.

Ferrous Reducing Power Assay

The method described by (Oyaizu, 1986; Do QD *et al*., 2014) [17-18] was used to evaluate the reducing power capacity. The test tubes were used to take the different solutions into. Then 0.625 ml of potassium buffer (0.2 M) and 0.625 ml of potassium ferricyanide [$K_3Fe(CN)_6$], (1%) solution were added into each of the test tubes. After that the reaction mixtures were incubated for 20 minutes at 50°C to complete the reaction. 0.625 ml solution of 10% trichloro- acetic acid (TCA) was added into each of the test tubes. Then the total mixtures were centrifuged at 3000 rpm for 10 min. The supernatant (1.8 ml) was taken out of the mixture and mixed with the water (1.8 ml). Then 0.36 ml solution of 0.1% ferric chloride ($FeCl_3$) was added to the diluted reaction mixtures. The solution's absorbance was measured using a spectrophotometer at 700 nm. Under the same conditions as the rest of the samples solution, a typical blank solution contained the same mixture without plant extract or standard. The absorbance of the blank solution was measured against a solvent. Reducing power was indicated by increased absorbance of the reaction mixture.

DPPH Radical Scavenging Activity

The free radical scavenging capacity of different extractives was evaluated with the help of the DPPH (1, 1- diphenyl- 2-picrylhydrazyl) (Choi *et al*.; 2000, Karadag A *et al*., 2009) [19-20]. Methanol solutions (1ml) of different standards were taken into the test tubes. Each of the test tubes was given a small amount (2.4 ml) of the methanol solution of DPPH (0.004%

W/V). The test tubes were then incubated at RT for 30 minutes in dark place to complete the reaction. The solution's absorbance was measured using a spectrophotometer at 517 nm. The following equation was used to calculate the percentage of inhibition activity.

$$\% I = \{(Ao - A1) / Ao\} \times 100$$

Where, Ao is the absorbance of the control and, A1 is the absorbance of the extract/standard. The % of inhibition was plotted against concentration and IC_{50} was calculated from the graph.

Results

Calibration curve of different standards are given in table-1.

Table 1: Calibration curve of different standards

Standards	Conc. range	Calibration Curve	R^2
Gallic acid (GA)	2.5-30µg/ml	y =0.0099x+0.0003	0.9891
Catechin (CA)	2.5-30µg/ml	y=0.0071x+0.0074	0.9834
Ascorbic acid (AA)	12.5-200µg/ml	y=0.0015x+0.0135	0.9808

Determination of Total Phenolic Content

Total phenolic content of *Trewia nudiflora* leaf, peel and flesh of both methanol and ethyl acetate extract was determined by using the Follin-ciocalteu reagent (FCR). Based on the standard curve of GA (Gallic Acid), the amount of phenolics in extractives was determined, as indicated in the table. In terms of dried extractives, the results were represented as mg of GAE/gm. The results of phenolic content of extracts of leaves, peel and flesh is shown in the table-2.

Table 2: Determination of Total Phenolic content of *Trewia nudiflora* leaf, peel and flesh of both methanolic and ethyl acetate extract

Sample	Average Phenolic Content (mg of GAE /g)±Standard Deviation (SD)
LTNM	14.537±0.696
LTNE	2.345±0.175
PTNM	10.166±0.699
PTNE	7.809±0.640
FTNM	5.975±0.271
FTNE	14.298±0.680

Gallic Acid Equivalent (GAE) was used to quantify the total phenolic content. Table-2 shows that the methanolic extract of the leaf of *Trewia nudiflora* (LTNM) possesses the highest level of phenolic content among the methanol and ethyl acetate extracts (14.537±0.696 mg of GAE/g of extract). The flesh of *T. nudiflora* (FTNE) had the highest phenolic content of any ethyl acetate extracts (14.298±0.680 mg of GAE/gm). Peel extract in methanol (PTNM) likewise has a high phenolic content (10.166±0.699 mg of GAE/g). The least quantity of phenolic content (2.345±0.175 mg of GAE/g) is found in ethyl acetate leaf extract (LTNE).

Determination of Total Flavonoids

Total flavonoid content of *Trewia nudiflora* leaf, peel, and flesh was assessed in methanol and ethyl acetate extracts by using the aluminum chloride colorimetric method. According to the table, CA ((+)-Catechin) standard curve was used to determine the flavonoid content of extractives. In terms of dried extractives, the results were represented as mg of CAE/gm. Table-3 summarizes the findings of the flavonoid level of ethyl acetate and methanolic extracts of leaves, peels, and flesh.

Table 3: Determination of Total Flavonoid content of *Trewia nudiflora* leaf, peel and flesh of both methanolic and ethyl acetate extract

Sample	Average Flavonoid Content (mg of CAE/g)±Standard Deviation (SD)
LTNM	5.398±0.414
LTNE	5.135±0.574
PTNM	3.004±0.059
PTNE	3.858±0.285
FTNM	2.290±0.042
FTNE	4.633±0.867

Total Flavonoid Content was measured as Catechin Equivalent (CAE). From the table-3 it was observed that among the extracts of methanol and ethyl acetate, methanolic extract of leaf of *Trewia nudiflora* (LTNM) possesses the maximum level of flavonoid content (5.398±0.414 mg/g of CAE).

Among the extracts of ethyl acetate leaf of *T. nudiflora* (LTNE) possesses the maximum amount of flavonoid (5.135±0.574 mg of CAE/g). Ethyl acetate extract of flesh (FTNE) also contains prominent amount of flavonoid content (4.633±0.867 mg of CAE/g) followed by (PTNE) ethyl acetate extract of peel (3.858±0.285 mg of CAE/g). The least quantity of flavonoid content (2.290±0.042 mg of CAE/g) is found in methanolic extract of flesh (FTNM).

Determination of Total Antioxidant Capacity

The total antioxidant content of *Trewia nudiflora* leaf, peel, and flesh was determined in both methanol and ethyl acetate extract by using the phosphomolybdenum method. According to the table, the amount of total antioxidants in extractives was determined using the standard curve for ascorbic acid (AA). The results were given in mg of AAE/gm of dried extractives. Table-4 displays the findings of the Total Antioxidant Capacity of methanolic and ethyl acetate extracts of leaves, peels, and flesh.

Table 4: Determination of Total Antioxidant Capacity of *Trewia nudiflora* leaf, peel and flesh of both methanolic and ethyl acetate extract

Sample	Average Antioxidant Capacity (mg of AAE/g)±Standard Deviation (SD)
LTNM	29.311±0.429
LTNE	15.311±1.516
PTNM	23.889±0.555
PTNE	20.467±5.549
FTNM	14.111±0.847
FTNE	23.889±1.997

Total Antioxidant Capacity of *Trewia nudiflora* leaf, peel and flesh of both methanolic and ethyl acetate extract was measured as Ascorbic Acid Equivalent (AAE). From the table-4 it was observed that among the extracts of methanol and ethyl acetate methanolic extract of leaf of *Trewia nudiflora* (LTNM) contains the highest amount of Antioxidant Capacity (29.311±0.429 mg of AAE /g).

Methanolic extract of peel of *T. nudiflora* (PTNM) and ethyl acetate extract of flesh of *T. nudiflora* (FTNE) both contains the second highest amount of Antioxidant Capacity (23.889±0.555 mg of AAE/g and 23.889±1.997 mg of AAE/g respectively). Ethyl acetate extract of peel (PTNE) also contains prominent amount of Antioxidant component (20.467±5.549 mg of AAE/g) followed by (LTNE) ethyl acetate extract of leaf (15.311±1.516 mg of AAE/g). Methanolic extract of flesh (FTNM) contains the lowest

amount of Antioxidant component (14.111±0.847 mg of AAE/g).

Ferrous Reducing Power Assay

A compound's reducing ability serves as an important marker of potential antioxidant activity. In this test, the yellow color of the test assembly changes to various shades of green and blue depending on the reducing power of the antioxidant sample. The results of reductive capabilities of Methanolic extracts of leaves, peel and flesh and AA standard is shown in the table-5.

Table 5: Reductive capabilities of Methanolic extracts and AA standard

Sample	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200µg/ml
Ascorbic acid	0.492	0.551	0.625	0.689	0.790
LTNM	0.192	0.253	0.393	0.626	0.760
PTNM	0.148	0.149	0.171	0.218	0.306
FTNM	0.163	0.146	0.167	0.203	0.298

The Reducing Power Capacity was compared against Ascorbic Acid (AA) standard. Methanolic extracts of leaf, peel and flash has appreciable reducing power. All have lower reducing power than the standard Ascorbic Acid (AA). The capacity for reducing power started growing as concentration levels did as well. At a concentration of 200 µg/ml, leaf of methanolic extract (LTNM) has the most reducing power. The results of reductive capabilities of Ethyl Acetate extracts of leaves, peel and flesh and AA standard are shown in the table-6.

Table 6: Reductive capabilities of Ethyl Acetate extracts and AA standard

Sample	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200µg/ml
Ascorbic acid	0.492	0.551	0.625	0.689	0.790
LTNE	0.136	0.132	0.144	0.159	0.186
PTNE	0.145	0.148	0.176	0.242	0.418
FTNE	0.174	0.241	0.307	0.519	0.765

The Reducing Power Capacity was compared against Ascorbic Acid (AA) standard. Ethyl Acetate extracts of leaf, peel and flash has appreciable reducing power. All have reducing power lower than the standard Ascorbic Acid (AA). The capacity for reducing power grew as concentration levels did as well. Among the ethyl acetate extracts, the flesh of ethyl acetate extract (FTNE) has the highest reducing power at various concentrations. The methanolic extract of the leaf of *Trewia nudiflora* (LTNM) demonstrated highest Reducing Power Capacity of all the ethyl acetate and methanol extracts. Ascorbic Acid (AA), the reference standard, and extracts decrease activity in the following order:

AA >LTNM > FTNE > PTNE > PTNM > FTNM > LTNE

DPPH Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is commonly used to assess the ability of antioxidants to scavenge free radicals. The antioxidant activity of *Trewia nudiflora* extract was evaluated by the widely used and most reliable DPPH scavenging assay method. Table-7 shows the scavenging activity of the methanol extracts from leaves, peel and flesh and the results for the BHT standard.

Table 7: Percentage of free radical scavenging activity of methanolic extracts

Sample	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
BHT	28.182	52.657	65.664	77.483	83.427
LTNM	24.196	39.091	62.727	69.790	72.517
PTNM	21.119	22.867	29.301	40.490	63.986
FTNM	19.021	20.769	26.853	36.014	55.594

Among all the methanolic extract the leaf (LTNM) showed the highest percentage of scavenging activity which was the closest to the standard BHT. The second highest scavenging activity was shown by the peel (PTNM) which was followed by the flesh of methanolic extract of *T. nudiflora* (FTNM). In all the cases percentage of scavenging activity increased with the increase of concentration. The results of DPPH radical scavenging activity of ethyl acetate extracts of leaves, peel and flesh and BHT standard is shown in the table-8.

Table 8: Percentage of free radical scavenging activity of Ethyl Acetate extracts

Sample	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
BHT	28.182	52.657	65.664	77.483	83.427
LTNE	12.237	11.888	12.378	15.734	20.629
PTNE	16.364	17.483	25.315	37.343	55.594
FTNE	19.720	29.790	46.573	57.343	70.909

The flesh (FTNE) showed the highest percentage of scavenging activity among all the Ethyl Acetate extracts, which was comparable to the standard BHT. The second highest scavenging activity was shown by the peel (PTNE) which was followed by the leaf of ethyl acetate extract of *T. nudiflora* (LTNE). The percentage of scavenging activity increased with increasing concentration. Among all the extracts of methanol and ethyl acetate methanolic extract of leaf of *Trewia nudiflora* (LTNM) showed the highest percentage of scavenging activity (72.517% at 200 µg/ml concentration). The percentage of scavenging activity for both PTNE and FTNM was the same at a concentration of 200 µg/ml. Following is the order in which the percentage of scavenging activity of extracts and the reference standard BHT decreases:

BHT > LTNM > FTNE > PTNM > PTNE > FTNM > LTNE

The IC_{50} value of different extracts was calculated from the graph using excel sheet. The values of IC_{50} of different extracts are given in the table-9.

Table 9: IC_{50} value of different extracts and BHT standard

Sample	IC_{50} µg/ml
BHT	27.8
LTNM	40.95
LTNE	816.04
PTNM	140.04
PTNE	169.77
FTNM	172
FTNE	65.81

Among all the extracts of methanol and ethyl acetate leaf of methanolic extract (LTNM) has the lowest IC_{50} value (40.95 µg/ml) which is the closest to the IC_{50} value of BHT standard (27.76 µg/ml). This indicates that leaf of methanolic extract (LTNM) has very prominent free radical scavenging activity or antioxidant activity. The flesh of ethyl acetate extract (FTNE) also has significant free radical scavenging activity.

The ethyl acetate extract of flesh (FTNE) has an IC_{50} value (65.81 µg/ml). The peel part also showed significant IC_{50} value. The ethyl acetate extract of leaf had a higher IC_{50} value than the other extracts, indicating that it had negligible free radical scavenging activity.

Discussion

A preliminary phytochemical screening of *Trewia nudiflora* leaves and fruits revealed the presence of a variety of phytochemicals, including flavonoids, steroids, glycosides, phenolic compounds, alkaloids, and tannins [21]. In comparison to standard ascorbic acid, *Trewia nudiflora* roots and leaves showed significant antioxidant activity in ethanol and aqueous extracts [8]. Because it was established that plants containing phenolic content, flavonoid content and antioxidant activity exerts antiarthritic [22], anti-cancer [23], antibacterial [24] and antidiabetic activities [25-26].

In our study, we found that the methanolic extract of *Trewia nudiflora* leaves (LTNM) contained the highest phenolic content (14.537±0.696 mg of GAE/g) among the extracts of methanol and ethyl acetate. The flesh of T. nudiflora (FTNE) had the highest phenolic content of all the ethyl acetate extracts (14.298±0.680 mg of GAE/g). Methanol extract of peel (PTNM) and ethyl acetate extract of peel (PTNE) also contained prominent amount of phenolic content. Thus, the results showed that our plant extracts were a significant source of phenolic compounds and natural antioxidants as claimed in the previous study. In our study, methanolic extracts of *Trewia nudiflora* leaves (LTNM) were found to have the highest flavonoid content when compared to methanol and ethyl acetate extracts (5.398±0.414 mg of CAE/g). The methanol and ethyl acetate extracts of peel and flesh also contained flavonoids. Among them ethyl acetate extract of flesh (FTNE) contained prominent amount of flavonoid content (4.633±0.867 mg of CAE/g). Thus, our plant parts are significant source of flavonoid. Due to their antioxidant and anti-inflammatory features, these plant parts can therefore act as an important source of flavonoid for anticarcinogenic and antimutagenic activities, as claimed in the previous study [27-28].

In our study, Total Antioxidant Capacity of *Trewia nudiflora* leaf, peel and flesh of both methanolic and ethyl acetate extract was measured as Ascorbic Acid Equivalent (AAE). It was observed that among the extracts of methanol and ethyl acetate methanolic extract of leaf of *Trewia nudiflora* (LTNM) contained the highest amount of Antioxidant Capacity (29.311±0.429 mg of AAE/g). Methanolic extract of peel of *T. nudiflora* (PTNM) and ethyl acetate extract of flesh of T. nudiflora (FTNE) both contained the second highest amount of Antioxidant Capacity (23.889±0.555 mg of AAE/g and 23.889±1.997 mg of AAE/g respectively). The results of our study also showed that our plant parts possessed significant amount of natural antioxidants (Table-4). Therefore, our plant extracts may work very well to prevent the harmful effects of oxidative stress.

According to earlier research, phenols, a significant class of antioxidant phytochemicals, play a significant role in biology and free-radical scavenging [29]. The total phenolic content (TPC), antioxidant (AOA), and free radical scavenging activities (FRSA) of some plant extracts were investigated to determine their potential sources, followed by the specific phenolic composition [30]. AOA ranged from 24.2% (*Curcuma zeoderia*, leaves) to 96.9% (*Trewia nudiflora*, leaves) and TPC ranged from 2.8 mg/g (*Withania somnifera*, roots) to 107.8 mg/g (*Cassia fistula*, fruits) [31].

In our study, Reducing Power Capacity increased with the increase of concentration. Among all the extracts of methanol and ethyl acetate methanolic extract of leaf of *Trewia nudiflora* (LTNM) showed the highest Reducing Power Capacity. Flesh of ethyl acetate extract (FTNE) has also a prominent amount of reducing power at different concentration. The results of our Reducing Power Capacity assay indicates that this plant parts can act as a significant source of antioxidant component as mentioned in the following previous study. Ascorbic Acid (AA), the reference standard, and extracts decrease activity in the following order:

AA> LTNM > FTNE > PTNE > PTNM > FTNM > LTNE

In a previous study *A. indica* bark, *Cinnamomum zeylanicum*, *Casuarina equisetifolia*, *Lawsonia inermis* fruits, *Indigofera tinctoria* flowers, and fruits and leaves of *T. nudiflora* all showed reducing power values between 0.6 and 2.8 ascorbic acid equivalents (ASE/ml) and very low inhibitory concentration values between 140 and 260 µg/ml, indicating the potential for these plant parts. Additionally, they demonstrated superior deoxyribose degradation inhibition compared to the reference standard [31].

The DPPH has been frequently used to assess an antioxidant's ability to scavenge free radicals. The IC_{50} value in our study, with the exception of the leaf ethyl acetate extract, ranged from 40.95 to 172 µg/ml. This suggests that our extracts have a significant FRSA when compared to the earlier study.

The percentage of scavenging activity increased with increasing concentration. Among all the methanolic extract the leaf (LTNM) showed the highest percentage of scavenging activity (72.517%) which was the closest to the standard BHT (83.427%). Among all the Ethyl Acetate extract the flesh (FTNE) showed the highest percentage of scavenging activity (70.909% at 200 µg/ml concentration) which was also close to the standard BHT. The percentage of scavenging activity of extracts and the reference standard BHT decreases in the following order:

BHT > LTNM > FTNE > PTNM > PTNE > FTNM > LTNE

With an IC_{50} value of 35.51 µg/ml, 39.93 µg/ml, 49.29 µg/ml, and 67.23 µg/ml respectively, compared to the standard ascorbic acid at 45.78 µg/ml, another previous study found that the ethanolic extracts of twigs, seeds, fruit, and leaves showed antioxidant activity [32]. The leaf of methanolic extract (LTNM) in our study had the lowest IC_{50} value (40.95 µg/ml), which was comparable to the IC_{50} value of the BHT standard (27.8 µg/ml). The ethyl acetate extract of flesh (FTNE) has also an IC_{50} value (65.81 µg/ml). This indicates that leaf of methanolic extract (LTNM) and flesh of ethyl acetate extract (FTNE) has very significant free radical scavenging activity. Low IC_{50} values indicate high free radical scavenging activity and vice versa. Our plant parts have prominent free radical scavenging capacity compared to the previous study.

Conclusion

In conclusion, we can say that our consequences in addition help the view that the medicinal plant *T. nudiflora* is a promising source of natural antioxidants. For leaf methanolic extract showed prominent result which indicates leaf part contained more polar compounds. The extract of leaf had given the most significant phytochemical and antioxidant activity in all the cases. In comparison to solvent in case of fruit parts ethyl acetate extracts of fruit parts had given more significant results compared to the methanolic extract which showed it contained less polar compounds. Peel part had showed an average antioxidant activity irrespective of plant

parts and solvent. Further studies are needed to determine the pharmacological activity of plant parts. According to this study, the extracts may be used as a source of nutraceuticals or natural antioxidants to treat degenerative diseases and reduce oxidative stress. It is safe to use plant derived products because in developing countries there is high risk of misuse of synthetic antibiotics [33]. Further investigation seems necessary to isolate the active compounds in order to evaluate its various pharmacological activities in animals and humans.

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