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Anti-bacterial, Anti-oxidant and other Phytochemical Properties of *Datura innoxia* Mill. leaves

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Abstract

To investigate the phytochemicals present in the leaves of *Datura innoxia* Mill. and to assess its antioxidant and antibacterial properties in different organic solvents, leaf extracts were exposed to different standardized techniques. Folin–Ciocalteu method and Aluminium chloride method proved that the ethanolic extract has maximum phenolic content (72.35± 0.52 mg GAE/g) and flavonoid content (29.21± 1.25 mg EQ/g) respectively. The highest DPPH radical scavenging activity with IC_{50} value 91.398 µg/ml also was in the ethanolic extract as compared to methanol, hexane and chloroform extracts. Free radical scavenging and antioxidant property of extracts were observed in the sequence of ethanol>methanol>hexane>chloroform. There was a strong correlation between antioxidant activity with total phenolic (DPPH, R^2 = 0.41; PPM, R^2= 0.25) and total flavonoid contents (DPPH, R^2 = 0.39; PPM, R^2=0.23). Ethanolic and methanolic extracts showed antibacterial potential against the tested pathogenic strains; *Staphylococcus aureus* and *Escherichia coli* with a zone of inhibition ranging between 16± 0.9 to 27.5±0.8 mm. This study has proved that ethanolic leaf extract of *D. innoxia* showed bacterial inhibition and antioxidant activities and this herb can be assessed as a potential therapeutic species.

Keywords

Datura innoxia, IC 50, Kirby-Bauer test, Phytochemical screening, antioxidant activity.

Introduction

In the herbal medical plants, the Solanaceae family, consisting approximately of 2500 species, is placed at the top with its high ethnobotanical content which is extensively used by the modern scientific fraternity for the further exploration and invention of effective medicines (1–4). *Datura innoxia* is a widespread annual plant from the Solanaceae family. Therapeutically it is a rarely explored plant and even recommended as a species to be eliminated in certain parts of the world such as South Africa, the Pacific region and the Isabella Islands (5-7). Despite its bad reputation as a harmful plant due to its aggressive growth habit and poisonous components (8, 9). it can be decontaminated to produce medically beneficial compounds (9-11).

D. innoxia (Fig.1) is invasive in the Galapagos Islands, New Caledonia, China, Taiwan, Ethiopia, Namibia and South Africa. It is widely naturalized in the tropics and increasingly in temperate Europe and North America (12), but today so common in India (9-13). Naturally, it appears in barren fields, waste areas, sidewalks and fencerows (14). In the tropics, *D. innoxia* is a per-

Fig. 1. *Datura innoxia* from Amity University premises, Lucknow.

ennial species but seen as an annual in temperate regions (12), that grows to a height of 2-5 feet. The plant is covered in small, soft greyish hairs that give it a greyish appearance. It has an entire-edged ovate to elliptic leaves. The flowers are ten toothed white and trumpet-shaped (15), with a length of 4-6 inches. They grow straight at first, then tilt downward. It blooms from early July through late October (12). The fruit is an egg-shaped spiny capsule with a diameter of around 5 c m (12). *D. innoxia* and *D. metel* are related closely but can be identified as plant height were substantially greater in *D. innoxia* and with deeper leaf indentations, larger capsules, a wider corolla (when divided on one side and rolled out flat), a wider calyx and heavier and sturdier roots (16).

D. innoxia is a medicinal herb as it is used to treat psychosis, fevers with catarrh, diarrhea, infestations, lumps, lesions, bronchitis, asthma, pulmonary difficulties, impotency, malaria, hair loss and cutaneous infections (17 -19). Atropine, scopolamine, hyoscyamine, withanolides (lactones) and tropanes are the active factors in *D. innoxia* (20-22). Mostly they are explored as anti-inflammatory and anti-cancerous (23-25).

As phytocompounds play a major role in the medicinal properties of plants (26-27) the current study was designed to evaluate the impact of different solvents on phytochemical constituents, total phenolic and flavonoid content and antioxidants and antibacterial activities of leaves of *D. innoxia*. This study has aimed to find out the best therapeutical output of *D. innoxia* as an agent of natural antioxidant and antibacterial and to preserve it from getting massively destroyed.

Materials and Methods

Sample collection

Leaves of *D. innoxia* were collected from the surrounding places of Amity University Campus, Lucknow. The sample was submitted to the Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP) authenticated taxonomically and assigned with Accession No: 8348. The collected leaves were cleaned, disinfected, rinsed with distilled water, and then dried in shade. The plant leaves were then crushed with liquid nitrogen and preserved at -20 ^{0}C for further use. Extraction of powdered leaves was done by dissolving them in solvents such as ethanol, methanol, hexane, aqueous and chloroform (5g/50 ml). All the extracts were maintained at room temperature for 48 hrs. Whatman Filter paper No. 1 was used to filter the extracts. By evaporating solvents from all of the filtrates, crude extracts were created (28).

Phytochemical screening

Phytochemical analysis was carried out following standard procedures (29, 30) with modifications (31) for the determination of biologically active components of the leaf of *D. innoxia* using different solvents such as ethanol, hexane, aqueous, methanol and chloroform.

Test for Alkaloids (Wagner's Test)

Dilute HCl is used for dissolving the extracts, then mixed with Wagner's reagent, and the change in color as reddish-brown shows the occurrence of alkaloids.

Test for Glycosides (Modified Borntragers's Test)

Extracts were hydrolyzed with dilute HCl and then mixed with a few drops of $FeCl_3$ (10%). The mixtures were kept in boiling water for about 5 min and then extracted with an equal amount of benzene. The benzene layers were parted and treated with a solution of ammonia. The presence of glycosides is indicated by the development of a rose-pink tint in the ammonical layer.

Test for Saponins (Froth Test)

The extract was diluted with 5 ml of distilled water and agitated for 15 min for this test. The presence of saponins is determined by the development of a foam layer.

Test for Sterols (Salkowski's Test)

Extracts were dissolved in chloroform and then in concentrated H_2SO_4. The appearance of a golden yellow color shows the occurrence of sterols.

Test for Phenols (Ferric Chloride Test)

The presence of phenols was evaluated by treating the extracts with 10% $FeCl_3$ solution. The appearance of bluish -black color gave a positive result.

Test for Tannins (Gelatin Test)

Extracts were mixed in gelatin (1%) solution containing NaCl, and the content of tannins was determined by the formation of a white precipitate.

Test for Flavonoids (Alkaline Reagent Test)

Flavonoids are identified qualitatively by mixing the extract with NaOH solution. Development of deep yellow color, which turns colorless on the accumulation of dilute HCl gives a positive result.

Test for Diterpenes (Copper Acetate Test)

The presence of diterpenes was determined by adding a

small amount of 10% copper acetate to the diluted extracts, which produces an emerald green color.

Total phenolic compounds assessment

The Folin–Ciocalteu is a recognized, widely-used method for quantifying Total Phenolic Compounds (TPC) designed by Singleton and Rossi. The Folin-Ciocalteu technique was carried out with modifications of to determine the phenolic compounds quantitatively in different solvents. Thus, 100 (µl) of the extract are mixed with 500(µl) of Folin-Ciocalteu reagent (diluted to 10% in distilled water) and 400(µl) of disodium carbonate (Na2CO3) at 75 mg/ml are added to the reaction mixture. The absorbance was observed at 765 nm after 2 hrs of incubation at normal temperature. A calibration curve was created using a gallic acid dilution series under the same operating conditions. The test was done in triplicate, and the results were denoted as mg/g of gallic acid using the calibration curve with the equation; Y = 0.006x, R^2 = 0.9823, where Y was the absorbance and x was the gallic acid equivalent.

Total flavonoid compounds assessment: Aluminium chloride method

The aluminum chloride method (34, 35) is used to determine the Total Flavonoid Concentration (TFC) of leaf extract using various organic solvents. 500 (µl) of each extract are added to 1500 (µl) of 95 % methanol, 100 l (µl) of 10% (w/v) AlCl3, 100 (µl) of 1 M sodium acetate and 2.8 (µl) of distilled water. The mixture was mixed and incubated nearby 30 minutes at room temperature in the dark. The blank was made by replacing the extract with 95 % methanol and measuring the absorbance with a UV spectrophotometer at 415 nm.

The samples were evaluated in duplicate, and the findings were calculated using the calibration curve and represented in mg equivalent quercetin / g dry mass, using the equation:

$$Y = 0.0065x, R^2 = 0.9787$$

where Y was the absorbance and x quercetin equivalent.

In vitro antioxidant activity

DPPH method for free radical scavenging activity

The scavenging activity of free radicals was evaluated using a 0.002 % DPPH (2,2-diphenyl-1-picrylhydrazyl)solution in methanol (36). Different solvents were used to make extracts and standard (2.5 µg/ml). 1 ml of 0.002% DPPH solution is combined with 1 ml of each of the extract and standard concentrations individually. After being held in the dark for about 30 min, the optical density of these mixes was measured at 517 nm. Ascorbic acid is used as a positive control. The proportion of inhibition was calculated by comparing the test and control results. The percentage of free radical inhibition by DPPH was computed as follows:

Inhibition percentage (%) = (Blank - Sample / Blank) × 100.

where the sample is the absorbance of the test sample and the blank is the absorbance of the control reaction mixture excluding the test sample. The plot of inhibition percentage against concentration was utilized to obtain IC_{50} values, which reflected the concentration of extract that induced 50% neutralization of DPPH radicals (37).

Phosphomolybdate (PPM) test

The phosphomolybdate (PPM) test was also carried out to evaluate the antioxidant property of the extracts (38). Reagent comprised of H_2SO_4 (600 mM), NaH_2PO_4 (28 mM) and Aluminum molybdate was added to 1 ml of extract at doses (20-120 µg/ml) (4mM). The mixture was incubated for 90 min at 90 °C. At 695 nm, the absorbance was measured. The reference standard was ascorbic acid. The antioxidant capacity is measured in mg of ascorbic acid equivalent per gram of dry matter (mgAA / gMS). The antioxidant capacity was estimated using the following formula:

Percentage of inhibition (%) = (Blank - Sample / Blank) × 100.

Antibacterial activity

Diffusion assay

Inhibitory potential of the leaf of *D. innoxia* using ethanol, methanol, hexane, chloroform and water as solvents was observed using an agar well diffusion assay using different extracts against pathogenic strains (UTIs) of *S. aureus (*US-6151, US-6081, and US-6090) and of *E. coli* (UE-1992, UE-1993 and UE-1981) isolated from urine samples of patients. All isolates were obtained from Dr.Ram Manohar Lohia Institute of Medical Science, Lucknow.

As per the Kirby-Bauer test (39), bacterial susceptibility based on Zone of Inhibition was evaluated (< 12 mm (resistant); <13-14 mm (intermediate), and >15 mm (susceptible). Before testing, each bacterial isolate's stock cultures were kept by inoculating them on blood agar (HiMedia Laboratory Pvt. Ltd.) and McConkey's medium (HiMedia Laboratory Pvt. Ltd.) plates (40). However, inoculum for MIC testing was prepared by transferring 1 ml of the adjusted inoculum in 25 ml of sterilized distilled water, which gives $5x10^5$CFU/ml inoculum concentration, and was matched with the 0.5 McFarland turbidity standard (Tulip Diagnostics (P) Ltd.), which is equivalent to 1.5 x 10^8CFU/ml (41).

Bacterial inoculum was spread on Muller Hinton Agar No. 2 (Hi-Media Laboratory Pvt. Ltd.) plates and then 6 mm sized wells were inoculated with 100 µl of *D. innoxia* leaf extract. Incubated at 37 ^{0}C for 24 hr and Zone of Inhibition were kept under observation for further studies (42). Gentamicin(reference antibiotic) and DMSO (10%)were used as positive as well as negative controls.

Statistical analysis

The experiments were carried out in triplicate, with the findings presented as mean ± standard deviation. The IC_{50} values and Linear regression coefficient (R^2) were calculated using Microsoft Excel 2010.

Results and Discussion

Phytochemical screening

Phytochemical analysis determines the presence of bioactive compounds qualitatively and observed results are given in Table.1. While Ethanolic leaf extract of *D. innoxia* showed the occurrence of alkaloids, glycosides, saponins, sterols, tannins, phenol, flavonoids and diterpenes in hex-

Table 1. Phytochemical Analysis of different crude extracts of *D. innoxia* leaf. Ethanolic (E), Hexane (H), Aqueous (A), Methanolic (M) and Chloroform (C) extracts of *D. innoxia*. (+) denotes the presence and (+) indicates the absence.

Sl.No.	Phytochemical Tests	E	H	A	M	C
1	Alkaloids (Wagner's test)	+	-	+	-	+
2	Glycosides (Mod. Borntrager's test)	+	+	-	+	-
3	Saponins (Froth test)	+	-	+	-	+
4	Phytosterols (Salkowski's test)	+	-	+	-	-
5	Phenol (Ferric chloride test)	+	+	+	+	+
6	Tannins (Gelatin test)	+	-	+	-	-
7	Flavonoids (Alk. Reagent test)	+	+	+	+	+
8	Diterpenes (Copper acetate test)	+	+	+	+	-

ane extract Glycosides, phenol, flavonoids and diterpenes were prominent. In the aqueous extract, except glycosides, all other evaluated phytocompounds were recognized. However, glycosides, phenols and diterpenes were detected in the methanolic extract, whereas alkaloids, saponins, phenol and flavonoids were found in the chloroform extract. In a similar study, the presence of atropine, scopolamine, essential oils, saponins, flavonoids, phenols as well as glycosides were identified and noted the absence of tannins, coumarins and carboxylic acid (43). It was denoted the presence of alkaloids, carbohydrates, cardiac glycosides, tannins, flavonoids, amino acids and phenolic compounds (44).

Total phenolic compounds assessment

Phenolic compounds in plants with redox properties are a natural source of free radical scavenging (45) and are more soluble in polar organic solvents. TPC of *D. innoxia* leaves was analyzed through the Folin-Ciocalteu method using different solvents. Results were obtained from a calibration curve (y=0.0066x+0.001;R^2 = 0.9823), as shown in Fig. 2 and expressed in gallic acid equivalents (GAE/g) per g dry extract weight. TPC in ethanolic leaf extract (72.35± 0.52 mg GAE/g) was observed as the highest followed by aqueous (53.31±2.6 mg GAE/g), methanol (43.13 ±1.3 mg GAE/g), hexane (35.14 ±2.3 mg GAE/g) and chloroform (35.14± 0.9 mg GAE/g) extracts.

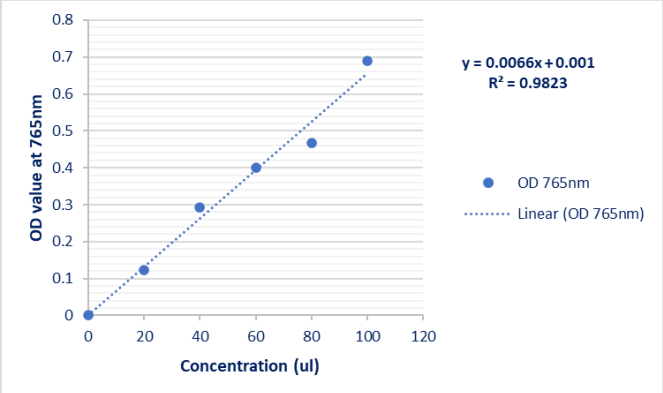

Fig. 2. Calibration curve to calculate total phenolic content from Folin-Ciocalteu method using gallic acid dilution from 0-100 µg/ml.

Total flavonoid compounds assessment

TFC were calculated from the calibration curve (y = 0.0065x + 0.001;R^2 = 0.9787), resulted from Aluminium Chloride method (Fig. 3) and expressed in mg of quercetin equivalents (EQ/g). Obtained datas show that the TFC was almost equal in all observed extracts such as ethanolic (29.21± 1.25 EQ/g), methanolic (17.95± 3.22 EQ/g), aqueous (20.92± 0.26

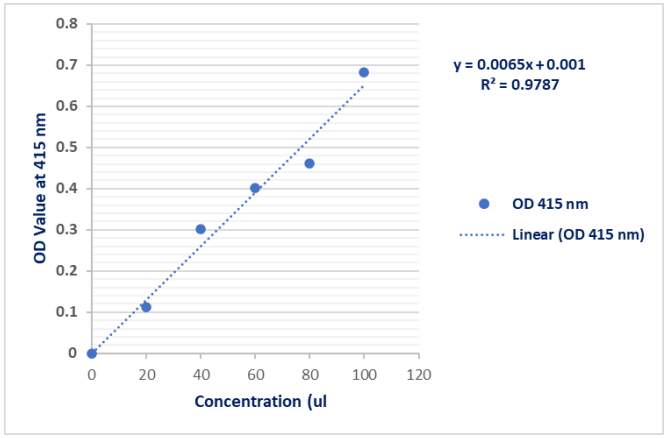

Fig. 3. Calibration curve to calculate total flavonoid content Aluminium chloride method using gallic acid dilution from0-100 µg/ml.

EQ/g), hexane (17.23 ±0.6 EQ/g) and chloroform (15.56± 0.25 EQ/g). In Fig. 4, TPC and TFC present in different solvents of *D. innoxia* leaves were comparatively analysed and these results are in agreement with other similar reports in

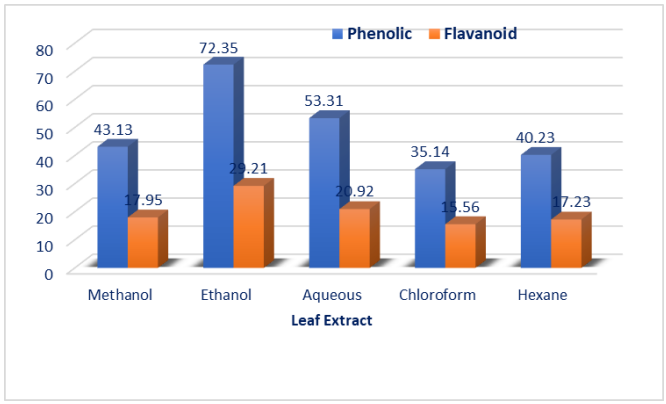

Fig. 4. Comparative analysis of total phenolic and flavonoid content of different extracts of *D. innoxia* leaf.

which it was pointed out; TPC as 30.97 ± 0.33 GAE/g and TFC as 15.13 ± 0.2 EQ/g (36) and noted TPC as 70.26 ± 1.12 mg GAE/g and TFC as 34.24 ± 1.28 EQ/g (46). Difference noted in results mostly due to variations in geographical features and extraction methods. Plants high in phenolic and flavonoid compounds are possible sources of natural antioxidants (48, 49).

Antioxidant activity- DPPH method

The DPPH radical scavenging method is associated with the reduction of DPPH in organic solution in the presence of a hydrogen-donating antioxidant, resulting in variation of color from purple to yellow at 517 nm (1, 38). Inhibition of free radical in percentage is calculated. DPPH radical scavenging activity of *D. innoxia* in different solvents is shown in Table. 2. In different solvents the scavenging effect was in the following order ethanol > methanol >hexane> Chloroform (Fig. 5) and it increased in a concentration-dependent manner. From DPPH results, The IC_{50} value was calculated through a linear regression graph using calibration equa-

Table. 2. Results of DPPH radical scavenging activity and % inhibition of different leaf extracts of *D. innoxia*. Values are represented as mean ± SD (n = 3)

Conc. of extract (µl)	% of Inhibition				
	Ascorbic acid	Ethanol extract	Methanol extract	Hexane extract	Chloroform extract
20	42.123 ±0.023	12.321±0.012	12.13±0.05	1.238± 8.46	2.3± 2.31
40	59.392±0.0125	21.112± 0.12	18.136±0.021	1.45±0.05	2.5±1.61
60	72.438± 8.46	36.432±0.271	27.882±0.002	32.14±0.0125	10.3±0.92
80	89.112± 0.53	45.128±0.02	32.11±0.112	46.17±0.023	16.78±2.77
100	92.112±0.012	52.132±0.27	39.211±0.5	64.98±0.05	25.78±3.21
IC_{50} µg/ml	39.52	91.398	124.87	135	213

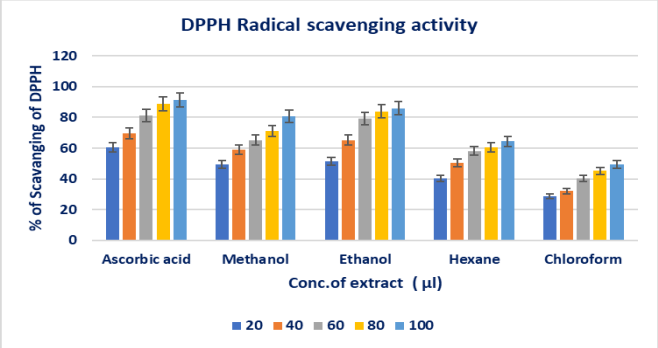

Fig. 5. Comparison of DPPH scavenging activity of ascorbic acid and different extracts of *D. innoxia* leaves. Results expressed as the mean ± standard deviation (n=3) at concentrations of 20, 40, 60, 80 and 100 µg/ml.

tion, Y= MX+C to establish the amount of the sample needed to inhibit 50% of radical. The antioxidant activity of the sample is considered higher with the lower IC_{50} values. According to one report (50), the extract that possesses an IC_{50} value from 10 to 50 µg/ml exhibit the highest antioxidant activity, 50-100 µg/ml exhibit minimum antioxidant activity and >100 µg/ml is considered to possess weak antioxidant activity (51, 52).

Highest antioxidant activity with IC_{50} value was recorded in ethanolic extract with a value 91.398 µg/ml (Y=0.53X +1.11, R^2=0.98) followed by methanolic extract with 124.87 µg/ml (Y=0.38X +2.59,R^2=0.98), hexane extract with 135 µg/ml (Y=0.42X -7.96,R^2=0.80) and chloroform extract with 213 µg/ml (Y=0.25X -3.25,R^2=0.90). These findings are in consistent with those of other similar studies, in which one study reported that DPPH radical scavenging activity of methanolic extract as 146.69 ± 8.46 µg/ml (43). However, ascorbic acid with a lower IC_{50} value with 39.52 µg/ml (Y=0.88X +15.29,R^2=0.91) has greater antiradical activity than the extracts (Fig. 6-10).

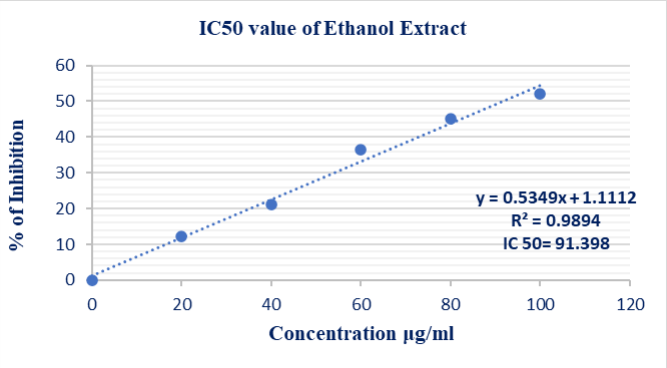

Fig. 6. Linear regression graph to calculate the IC $_{50}$ value that induced 50% neutralization of DPPH radicals at different concentrations of ethanol leaf extract of *D. innoxia* (0-100 µg/ml).

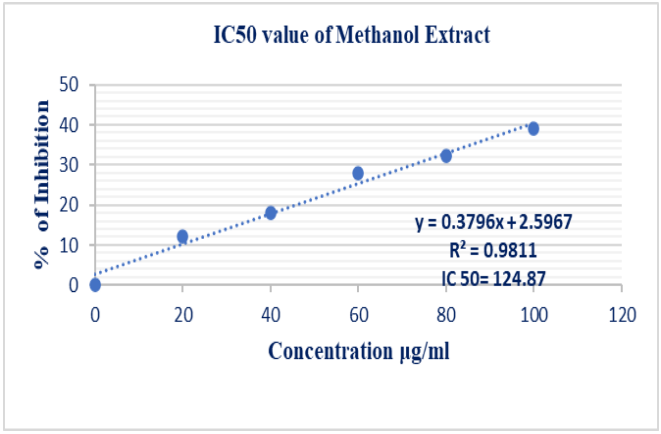

Fig. 7. Linear regression graph to calculate the IC $_{50}$ value that induced 50% neutralization of DPPH radicals at different concentrations of methanol leaf extract of *D. innoxia* (0-100 µg/ml).

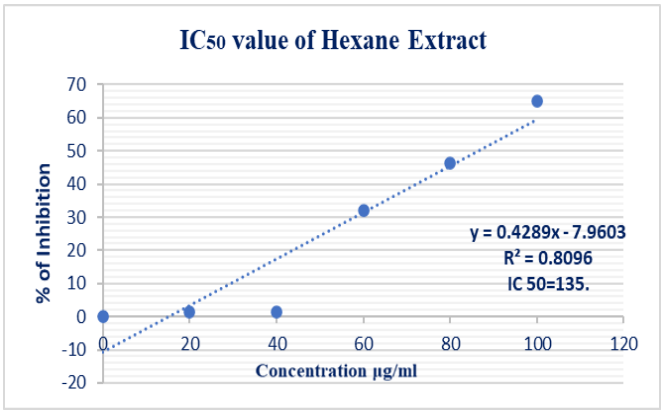

Fig. 8. Linear regression graph to calculate the IC $_{50}$ value that induced 50% neutralization of DPPH radicals at different concentrations of hexane leaf extract of *D. innoxia* (0-100 µg/ml).

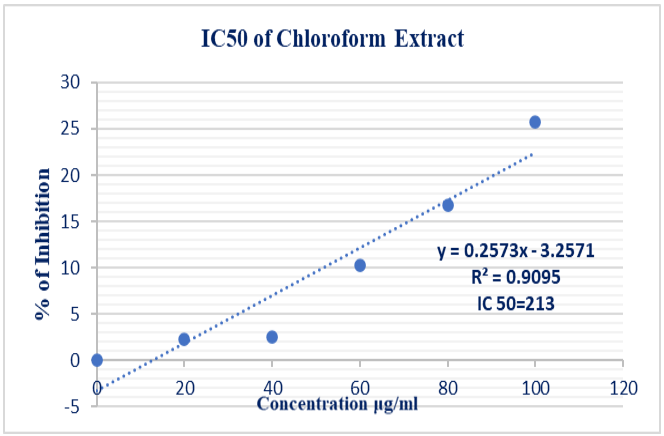

Fig. 9. Linear regression graph to calculate the IC $_{50}$ value that induced 50% neutralization of DPPH radicals at different concentrations of chloroform leaf extract of *D. innoxia* (0-100 µg/ml).

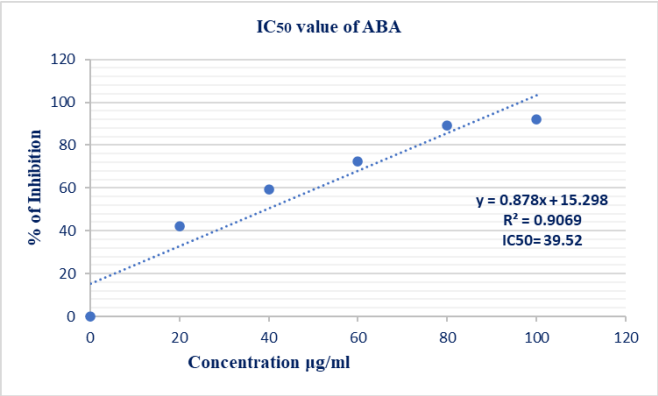

Fig. 10. Linear regression graph to calculate the IC $_{50}$ value that induced 50% neutralization of DPPH radicals at different concentrations of standard antioxidant (control); ascorbic acid (0-100 µg/ml).

Antioxidant activity- PPM method

The phosphomolybdate test causes the elimination of the phosphomolybdate ion in the presence of a scavenger, resulting in the formation of a green mixture of phosphate/MoV that may be measured spectrophotometrically (38). At 695 nm, the absorbance was measured and the antioxidant capacity of each solvent extract was measured in mg of ascorbic acid equivalent per g of dry matter (mgAA / gMS). The result showed that (Fig. 11) antioxidant activity in terms of percentage of inhibition was sequent; ethanol > methanol >hexane> Chloroform and it increased in a concentration-dependent manner. Fig. 12 pinpoints the comparative scavenging effect of leaf fractions on DPPH and PPM and justifies the antioxidant property. It highlights that the leaves of *D. innoxia* behave as antioxidants due to their hydrogen donating characteristics. However, ascorbic acid, a well known antioxidant utilized as a positive control, had higher antioxidant activity than *D. innoxia* extracts.

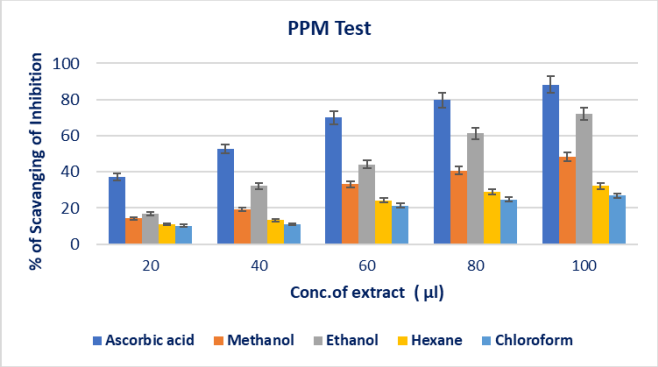

Fig. 11. Comparison of percentage of scavenging inhibition of ascorbic acid and different extracts of *D. innoxia* leaves resulted from PPM test. Results expressed as the mean ± standard deviation (n=3) at concentrations of 20, 40, 60, 80 and 100 µg/ml.

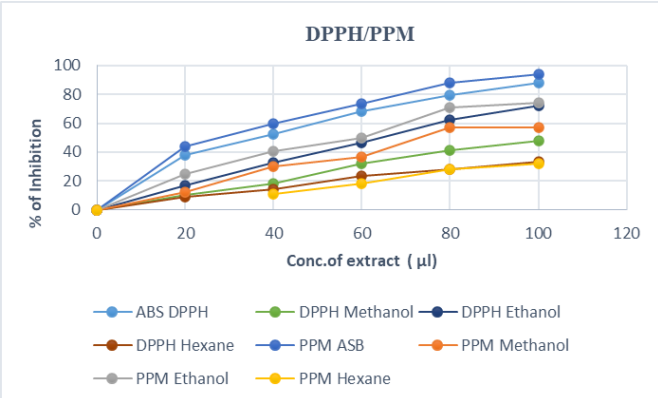

Fig. 12. Comparative analysis of different extracts (*D. innoxia* leaf) antioxidant activity and % of free radical inhibition resulted from DPPH and PPM methods.

A regression graph is used to calculate the correlation of total phenolic and flavonoid content with antioxidant capacity by using the mean value of antioxidant activity (%) at 50 mg/ml extract solution versus total phenolic and flavonoid. The correlation between antioxidant activity and total phenolic content showed a strong positive correlation with a correlation coefficient of 0.79 by DPPH method and 0.85 by PPM method (Fig. 13a) proved that there is a significant relationship between total phenolic content and antioxidant activity. The correlation between antioxidant activity and total flavonoid content also showed a strong positive correlation with a correlation coefficient of 0.73 by DPPH method and 0.62 by PPM method (Fig. 13b) proved that there is a significant relationship between total flavonoid content and antioxidant activity. This correlation is in agreement with the already proven result that extract with high total phenolic and flavonoid content (ethanolic extract) showed high radical scavenging activity (Fig. 5). Observations (36) also denoted that the antiradical activity is more in the ethanolic leaf extract of *D. innoxia* due to the highest presence of phenol and flavonoid.

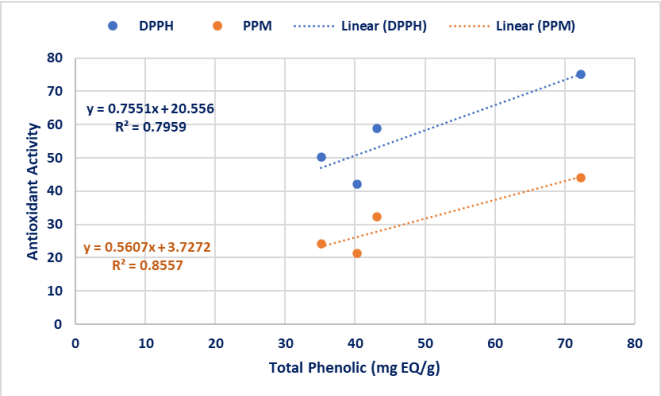

Fig. 13a. Graphs represent the mean value of antioxidant activity (%) at 50 mg/ml extract solution versus (a) total phenolic content and antioxidant activity. The correlation coefficient values for total phenolic (DPPH, R^2 = 0.79; PPM, R^2 = 0.85) was observed at a 95% confidence level.

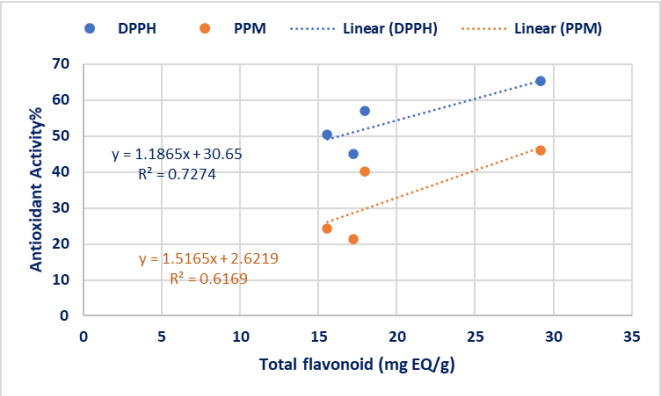

Fig. 13b. Graphs represent the mean value of antioxidant activity (%) at 50 mg/ml extract solution versus total flavonoid content and antioxidant activity. The correlation coefficient values for total flavonoid contents (DPPH, R^2 = 0.73; PPM,R^2=0.62) was observed at a 95% confidence level.

Antibacterial activity

Kirby-Bauer test denotes susceptibility based on Zone of Inhibition (ZOI) as < 12 mm (resistant); <13-14 mm

(intermediate) and >15 mm (susceptible). As shown in Fig. 14 ethanolic extract showed the highest ZOI against *S. aureus* US-6090 (27.5 mm), US-6051 (26.5 mm) and US-6081(25 mm); and the inhibitory activity is similar to Gentamicin (positive control). ZOI of hexane extract (US-6051 (25 mm), US-6081 (27 mm), US-6090 (25.5 mm)) exposed the fact that the pathogens are susceptible. Methanolic (US -6051 (14.5 mm), US-6081 (14 mm), US-6090 (12 mm)) and

Fig. 15. Zone of Inhibition (mm) of *E. coli* (UTIs) strains to show susceptibility to different extracts of *D. innoxia* leaf. DMSO and Gentamicin (reference antibiotic) were used as control.

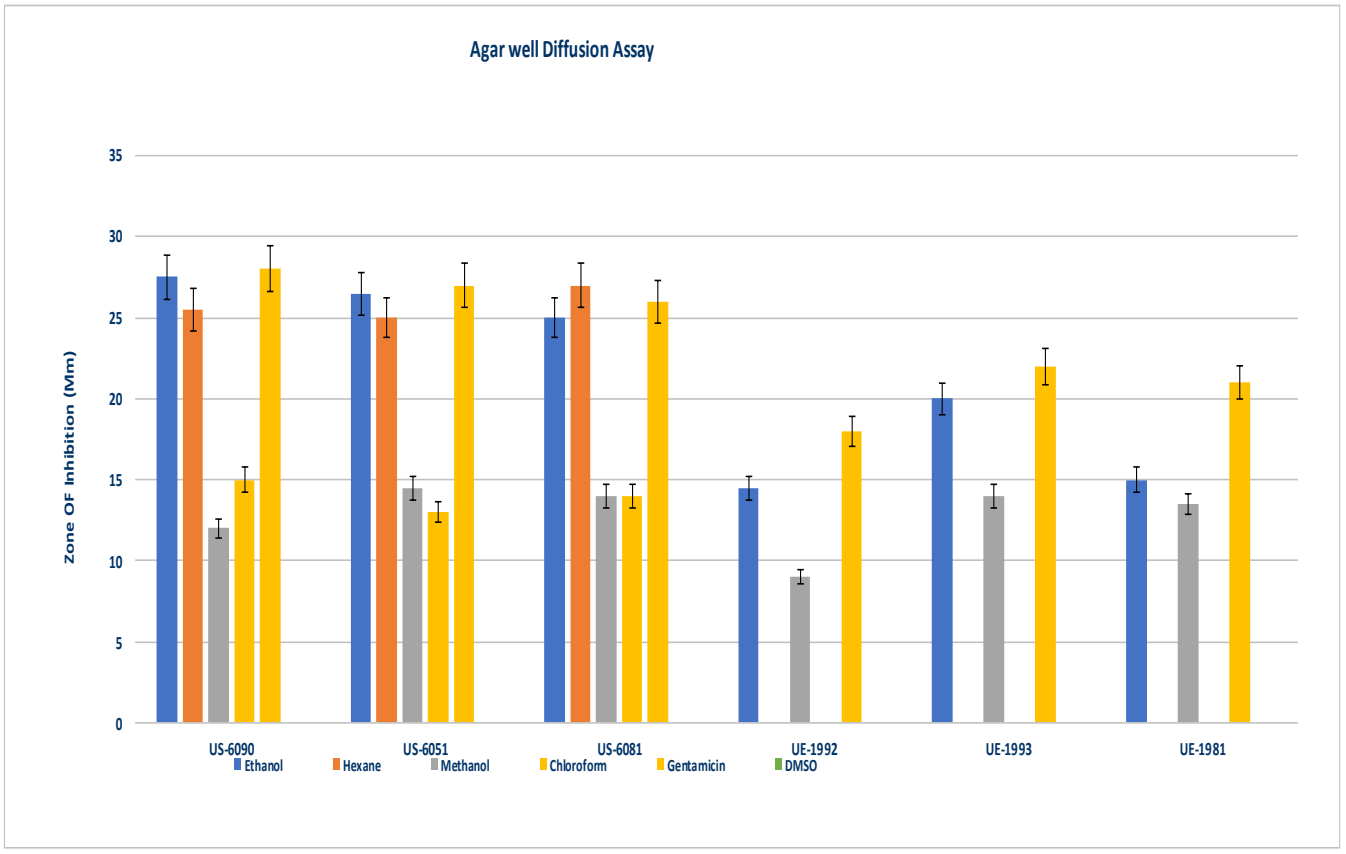

Fig. 16. Comparative analysis showing inhibitory activity of different extracts of *D. innoxia* leaf against *S. aureus* (US: 6090, 6051, 6081) and *E. coli* (UE:1992, 1993, 1981). DMSO and Gentamicin were used as control.

Fig. 14. Zone of Inhibition (mm) of *S. aureus* (UTIs) strains to show susceptibility to different extracts of *D. innoxia* leaf. DMSO and Gentamicin (reference antibiotic) were used as control.

chloroform (US-6051 (13 mm), US-6081 (14 mm), US-6090 (15 mm)) extracts revealed mild inhibitory effect against all the tested isolates.

E. coli pathogenic strains were susceptible to ethanolic extracts with ZOI of UE-1993 (20 mm) and demonstrated mild inhibition against UE-1992 (14.5 mm) and UE-1981 (15 mm). Moreover, U-1993 (14 mm), U-1981 (13.5 mm) isolates were found less effective, whereas U-1992, was found resistant towards the methanol extract. In addition, hexane and chloroform extracts displayed no activity against UE-1992, U-1993 and UE-1981 (Fig. 15).

The Agar diffusion assay provides significant evidence to broad-spectrum antibacterial property of leaf extract of *D. innoxia* against pathogenic *S. aureus* and *E. coli*. Comparative analysis indicated (Fig. 16) that the *S. aureus* was more susceptible than *E. coli*. It may be due to the cell wall complexity (53) of gram-negative bacteria. Ethanolic extract exhibits significant inhibition; comparatively closer to the activity of reference antibiotic (Gentamicin) against both the isolates. The higher antioxidant and phenolic property of ethanolic extract give a justification for this observation. The lesser anti-oxidant property of hexane extract may be one of the reasons for reducing its consistency in the result. At the same time, methanol extract with moderate antioxidant properties exhibited broad-spectrum inhibition. Which in turn confirm the co-relation between antibacterial and antioxidant properties. Solvent selection is crucial for the extraction of efficient bioactive compounds.

Qualitative and quantitative analysis of this study proved that ethanolic leaf extract of *D. innoxia* with its high rate of phenolic and flavonoid content as a good source of natural antioxidant and broad-spectrum antibiotic activity.

Conclusion

The qualitative and quantitative analysis confirmed the phytochemical richness of *D. innoxia*. Among the various solvents, the ethanolic leaf extract of *D. innoxia* exhibited the highest antioxidants that suppress free radicals in a dose-dependent manner with a strong positive correlation between antioxidant activity and total phenolic and flavonoid content. And its antibacterial property is similar to gentamicin the reference antibiotic. Therefore ethanol is recommended as the optimal solvent to obtain high content of phytochemicals as well as high antioxidants and antibacterial constituents from the leaves of *D. innoxia*. According to the data, phenolic acids and flavonoids may be the key contributors to antioxidative activities, giving this plant species strong broad-spectrum antibacterial properties. However, more research is needed to understand the bioactive components and antioxidant properties of these compounds in living models.

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Authors contributions

PM designed and co-ordinated the study and RG carried out the experimentation, statistical analysis and drafted the manuscript. Authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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