Section: Research Paper
ISSN 2063-5346

Extraction, Phytochemical Screening and Antioxidant Potential of Hydroalcoholic Extract of *Duranta Erecta* Leaves

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Abstract

Aim: This study aims to assess the therapeutic benefits and phytochemical properties of the Indian medicinal plant *Duranta erecta*. For millennia, traditional medical practices have used medicinal plants to treat, manage, and prevent a variety of illnesses. They contain a variety of bioactive substances, including as phenolic, alkaloids, flavonoids, and terpenoids, which have been proven to have a number of pharmacological effects, such as anti-inflammatory, antioxidant, antibacterial, and anticancer activity. A species of shrub or small tree in the Verbenaceae family is called *Duranta erecta*. It is also referred to by a number of other common names, such as sky flower, pigeon berry, and golden dewdrop. The medical benefits of *Duranta erecta* are well known, especially for its antioxidant activity.

Material and methods:

In this study, the authenticity of the chosen plant (*Duranta erecta*), extraction, phytochemical analysis, total phenol content, total flavonoid content, and antioxidant activity were all investigated. These methods included DPPH, Ferric chloride, and Hydrogen peroxide. Standard techniques were used for all of these operations.

Results: Vindhya Herbals in Bhopal provided authentication for the chosen plant material. Using the maceration method, the extraction was carried out using water and ethanol (a hydroalcoholic solvent).The findings of the phytochemical examination showed that the samples included a variety of bioactive substances, including terpenoids, phenols, alkaloids,

flavonoids, and flavonoids, among others. Due to its strong DPPH radical scavenging activity and total phenolic content, the hydroalcoholic extract of *Duranta erecta* leaves demonstrated a high antioxidant potential.

Conclusion: These findings suggest that *Duranta erecta* leaves could be a valuable source of natural antioxidants and warrant further investigation to explore their potential therapeutic applications.

Key words: *Duranta erecta*, Extraction, Phytochemical screening, Antioxidant

1. Introduction

People and communities in developing nations are increasingly turning to therapeutic plants. The usage of herbal medications has increased as a result of its affordability, accessibility, cultural acceptability, effectiveness, and rumoured lack of negative side effects as compared to synthetic treatments prevalent in Africa [1]. Plants generate a variety of bioactive compounds, many of which have medicinal benefits, making them a rich source of pharmaceuticals. Around 25% of prescription medications prescribed in the US come from plant origins [2]. The various elements give plants unique characteristics and characteristics. Plants are a significant source of natural therapies used to cure various ailments in traditional medicine.

The problem of preserving our rapidly deteriorating forest has come to light due to an overreliance on conventional folk medicine. The necessity for researchers to screen ornamental plants for biological and pharmacological qualities arises thus from the need to maintain our forests in order to support sustainable development. This is a component of research exploring additional uses for ornamental plants [3].

A beautiful plant, the golden dewdrop (D. erecta), grows untamed in arid coastal areas from a few metres above sea level to over 100 metre. It is primarily found in areas that are more humid, especially around roadways. D. erecta includes a variety of bioactive substances. A variety of phytoconstituents have been isolated from the D. erecta species' whole body, which has been used for phytochemical research. Steroids, flavonoids, terpenoids, triterpenes, C-alkylated flavonoids,beta-sitosterol,naringenin and saponins from triterpenes, steroidal glycosides are a few examples of significant phytochemical that have been reported. Numerous iridoid glycosides are also extracted from Duranta species to serve as durantosides [4]. Ten well-known substances, including bidesmosidic saponins, oleanolic acid, three phenylethanoids , and five flavonoids, were also extracted from D. erecta leaves, along with the two novel triterpene saponins durantanin IV (1) and V (2).

A number of diseases are healed using various plant components. (e fruit and leaves are used as vermifuges and diuretics in some situations, as well as being utilised to treat abscesses, intestinal worms, and malaria. It has been investigated that, D. erecta has potent antibacterial and anticancer properties [5]. It has several beneficial natural applications, including as insecticidal and antifungal effects [6].

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According to reports, a leaf extract in ethyl acetate significantly inhibited the growth of both Chloroquine-sensitive and Chloroquine-resistant Plasmodium falciparum strains. Aim of the current study is to carry out the Evaluation of Phytochemical and Antioxidant Potential of *Duranta Erecta L*.as a justification for its use in traditional medicine.

2. Materials and Methods

2.1 Material

In the month of September 2020 aerial portion of Duranta Erecta were harvested from Vindhya Herbals (MFP-PARC) Bhopal M.P. Gallic acid; 2, 2-diphenyl− 1-picrylhydrazyl hydrate (DPPH); Quercetin; Ascorbic acid; Folin-Ciocalteau phenol reagent (FCR) were from Sigma Aldrich Chemical Co.(Milwaukee,WI, USA) Bromocresol green; Lead acetate; Ferric chloride; Conc. Nitric acid; Copper acetate solution; Hcl Ethyl acetate; Sodium carbonate from Hi Media Laboratories Pvt. Ltd.(Mumbai India),Picric acid Fehling's A & B solutions; Sodium nitropruside;Chloroform; Ethanol Methanol;AlCl3 from SD Fine-Chem. Ltd (Mumbai India)

2.2 Methods

2.2.1 Procedure for extraction

Following procedure was adopted for the preparation of extract from the shade dried
And powdered stems

2.2.2 Maceration extraction technique [7]

The shade dried material was coarsely powdered before maceration extraction with petroleum ether. The extraction was maintained until the material had been defatting of the material had taken place. 80.63 gram of powdered leaves of *Duranta erecta* were exhaustively extracted using different solvent (Chloroform, Ethyl acetate, Ethanol and Aqueous) by maceration .The extract were evaporated above their boiling temperatures and kept in a air tight container free of contamination until they were utilsed. Finally the dried extracts % yields were computed.

2.2.3 Determination of percentage yield [8]

The percentage yield of yield of each extract was calculated by using formula:

$$\textbf{Percentage yield} = \frac{\textbf{Weight of extract}}{\textbf{Weight of powdered drug taken}} \textbf{x } \textbf{100}$$

2.2.4 Phytochemical screening [9]

Phytochemical examinations were carried out extracts as per the following standard methods.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium Hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of Saponins.

Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the occurrence of flavonoids.

7. Detection of proteins:

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formations of emerald green colour indicate the presence of diterpenes

2.3 Quantitative studies of phytoconstituents [10]

2.3.1 Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Parkhe and Bharti, 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15 second and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a UV/Visible spectrophotometer.

2.3.2 Estimation of total flavonoids content [11]

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

2.3.3 Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract

2.4 In-vitro antioxidant activity of extract of Duranta erecta using different methods

2.4.1 DPPH method:

DPPH scavenging activity was measured by the spectrophotometer (Parkhe and Jain, 2018). Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100µg/ml) was noted after 15 minutes. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. [12]

2.4.2 Ferric reducing ability of plasma (FRAP) assay

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide-ferric chloride method. Briefly, 0.2 mL of each of the extracts at different concentrations, 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1%) were mixed and incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide (Luqman *et al.*, 2012). The reaction was stopped by adding 2.5 mL of 10% (w/v) trichloroacetic acid followed by centrifugation at 1000 rpm for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance against the corresponding sample concentration [13]

2.4.3 Hydrogen peroxide scavenging activity [14]

In-vitro antioxidant activity of extract of *Duranta erecta* using hydrogen peroxide was performed as Czochra and Widensk (2002) proposed. Added 2ml hydrogen peroxide (43 mol) and 1.0 ml ethanolic sample [20-100 µl different extracts (4 mg / ml) ethanol] accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4). The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as

a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) ions has been calculated

3. Result And Discussion

Percentage yield of hydroalcoholic extract was found as shown in Table No 1.

The extract was subjected to preliminary phytochemical investigation. Various Phytochemical like Alkaloids, Glycosides, Flavonoids ,Phenol, Carbohydrates were discovered after Phytochemical screening as shown in Table No. 2.

Table No. 3 and 4 depicts presence of Gallic acid and Quercetin in herbal extract respectively. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.015x - 0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table No. 3 shows Total flavonoids content which was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y = 0.035x + 0.015, R^2=0.998, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table No. 5 depicts Total alkaloid content which was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: Y = 0.008x + 0.009, R^2=0.999, where X is the Atropine equivalent (AE) and Y is the absorbance.

Table No. 6 shows the hydrogen donating nature of extracts after using DPPH redical scavenging test. The inhibitory concentration 50 percent (IC50) value of Duranta Erecta hydroalcoholic extract was reported to be 75.84 g/ml when DPPH radical scavenging activity was compared to that of ascorbic acid (20.03 g/ml).

Table No. 7 shows ferric reducing capacity of extracts by using the potassium ferricyanide-ferric chloride method. The inhibitory concentration 50 percent (IC50) was found 89.72 g/ml with respect to ascorbic acid 20.84g/ml.

Table No 8 depicts *In-vitro* antioxidant activity of extract of *Duranta erecta* using hydrogen peroxide scavenging method. The IC50 value was reported to be 94.56 /ml when it was compared to that of ascorbic acid 35.98 g/ml.

Table 1: Percentage yield of hydroalcoholic extract of Duranta erecta

S.NO.	**Hydroalcoholic extract**	**% yield (W/W)**
1.	Leaves of *Duranta erecta*	8.62

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Table 2: Result of phytochemical screening of leaves of *Duranta erecta*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids (Hager's Test)	**-ve**	**-ve**	**+ve**	**+ve**
2.	Glycosides (Legal's Test)	**-ve**	**+ve**	**+ve**	**+ve**
3.	Flavonoids(Lead acetate Test)	**-ve**	**+ve**	**+ve**	**+ve**
4.	Diterpenes(Copper acetate Test)	**-ve**	**+ve**	**-ve**	**-ve**
5.	Phenol (Ferric Chloride Test)	**-ve**	**+ve**	**+ve**	**+ve**
6.	Proteins (Xanthoproteic Test)	**-ve**	**-ve**	**-ve**	**+ve**
7.	Carbohydrate(Fehling's Test)	**-ve**	**-ve**	**+ve**	**+ve**
8.	Saponins(Froth Test)	**-ve**	**-ve**	**-ve**	**+ve**

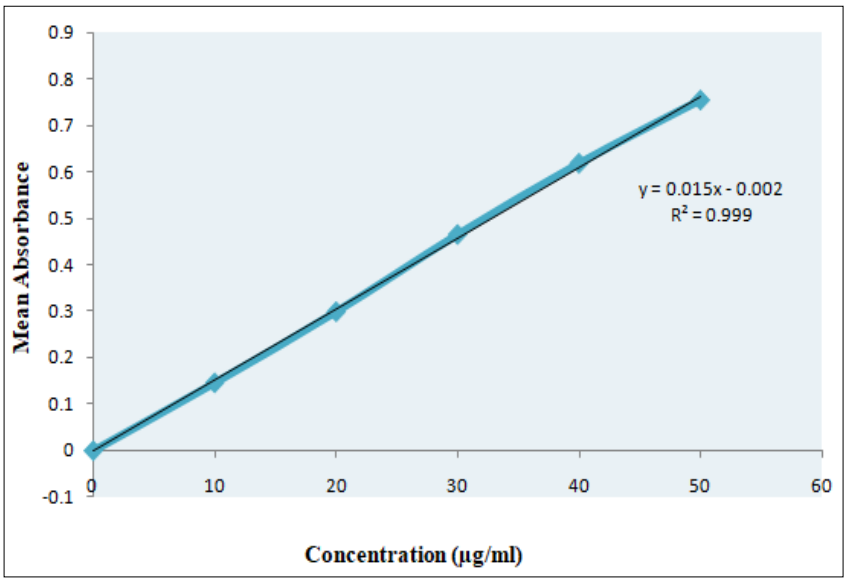
Total phenolics content estimation (TPC)

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.015x - 0.002$, $R^2 = 0.999$, where X is the gallic acid equivalent (GAE) and Y is the absorbance

Table 3: Results of estimation of total phenolic content

S. No.	Concentration (µg/ml)	Mean absorbance
1	10	0.146
2	20	0.299
3	30	0.465
4	40	0.618
5	50	0.754

Preparation of calibration curve of Gallic acid



Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **Y = 0.035x + 0.015, R^2=0.998**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 4: Results of estimation of total flavonoids content estimation (TFC)

S. No.	Concentration (µg/ml)	Mean absorbance
1	5	0.201±0.005
2	10	0.374±0.002
3	15	0.552±0.001
4	20	0.711±0.003
5	25	0.885±0.005

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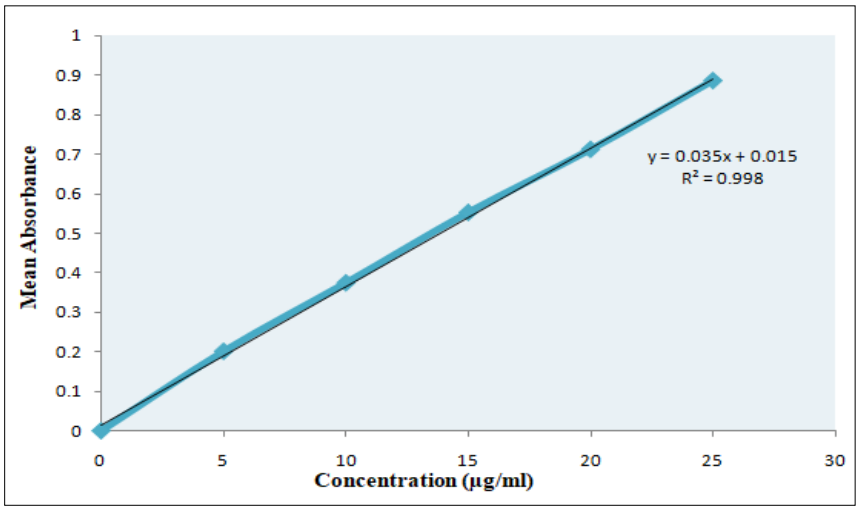
Preparation of calibration curve of Quercetin



Graph of Calibration curve of Quercetin

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: **Y = 0.008x + 0.009, R^2=0.999**, where X is the Atropine equivalent (AE) and Y is the absorbance.

Table 5: Results of estimation of total alkaloids content

S. No.	Concentration (µg/ml)	Mean absorbance
1	40	0.344
2	60	0.521
3	80	0.688
4	100	0.847
5	120	0.995

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Preparation of calibration curve of Atropine

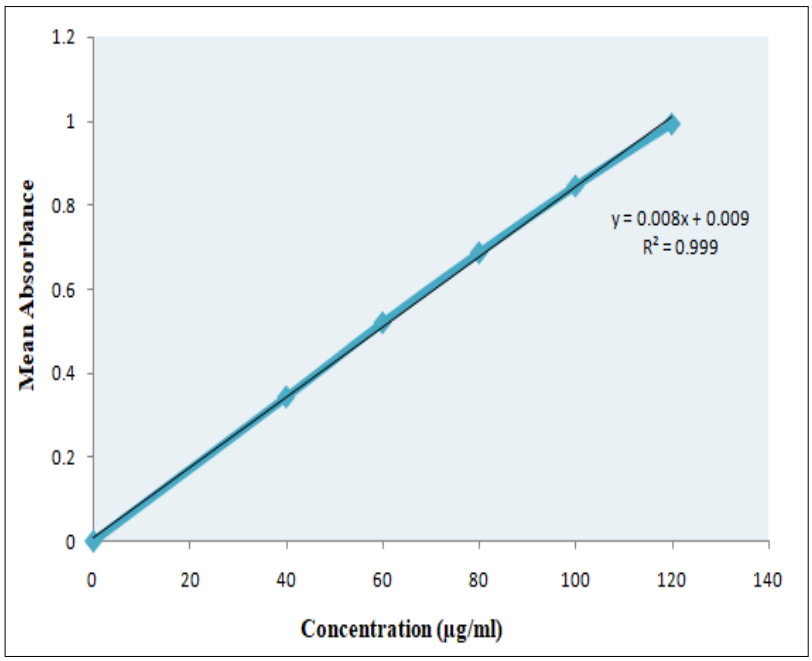

Table 6: Estimation of total phenolic, flavonoids and alkaloid content of leaves extract of *Duranta erecta*

S. No	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Chloroform	-	-	-
2	Ethyl acetate	0.235	0.412	-
3	Ethanol	0.869	0.911	0.245
4	Aqueous	0.547	0.645	0.122

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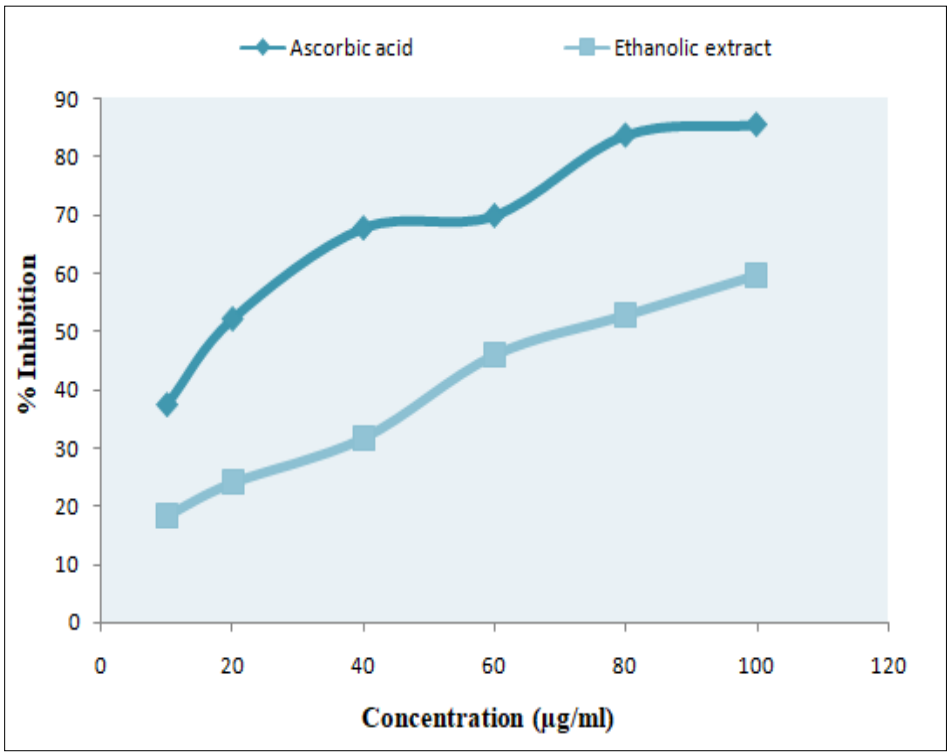
Results of DPPH method

Table 7: Estimation of total phenolic, flavonoids and alkaloid content of leaves extract of Duranta erecta

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
2	20	52.13	24.12
3	40	67.78	31.74
4	60	69.91	45.87
5	80	83.74	52.65
6	100	85.56	59.47
IC_{50}		20.03	75.84

% Inhibition of ascorbic acid and *Duranta erecta* extracts using DPPH



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% Inhibition of ascorbic acid and *Duranta erecta* extracts using DPPH method

Table 8: Result of % reducing power determination

Control	% Inhibition	
	Ascorbic acid	**Ethanolic extract**
10	41.49	9.85
20	52.58	16.74
40	56.69	20.59
60	67.78	28.96
80	71.88	35.87
100	73.56	51.34
IC_{50}	20.84	89.72

% reducing power determination of ascorbic acid and ethanolic extract of *Duranta erecta*

Reducing power activity of ethanolic extract with reference to ascorbic acid

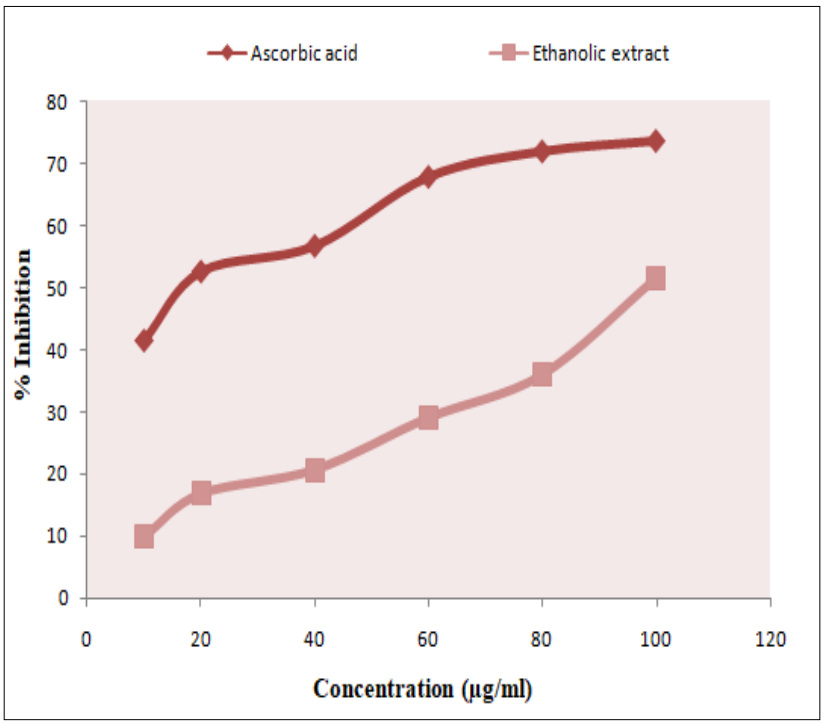

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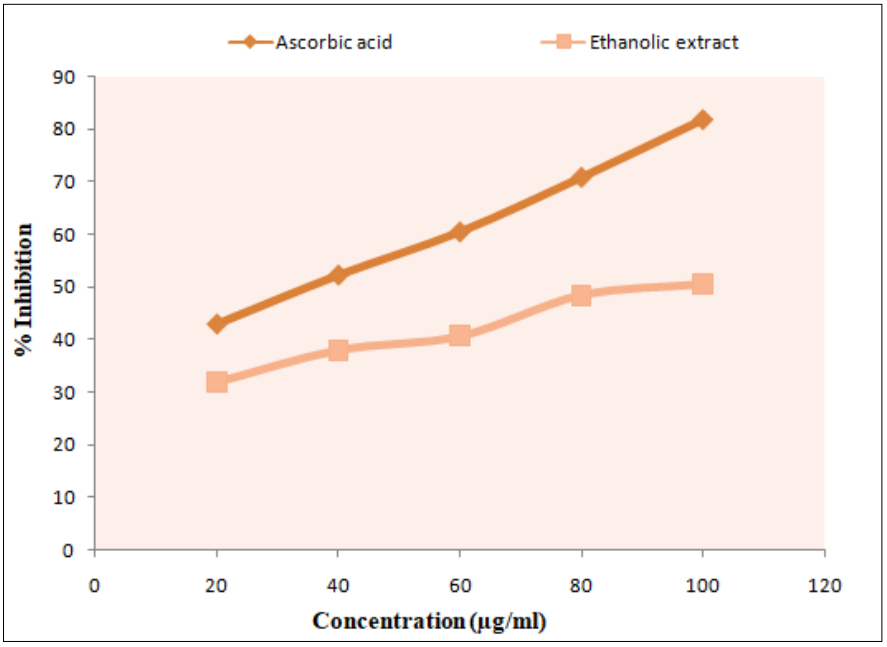
Table 9: Hydrogen peroxide scavenging activity

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	42.87	31.74
2	40	52.19	37.87
3	60	60.41	40.52
4	80	70.78	48.36
5	100	81.75	50.48
IC_{50}		35.98	94.56



% Inhibition of ascorbic acid and extract of *Duranta erecta* using hydrogen peroxide method

4. Conclusion

The results of present study clearly show that extract of *Duranta erecta* leaves contains a sufficient amount of significant phytochemical. For example, flavonoids, alkaloids, , phenolics, saponins, etc., which are essential sources of antioxidants. The several in vitro

antioxidant experiments (DPPH, FRAP, and H_2O_2 radical scavenging assay) revealed that the antioxidant activities of the extract of *Duranta erecta* leaves were dose dependant. According to the study's findings, *Duranta erecta* leaves may be a potential source of antioxidants. These Antioxidants may be extremely useful as therapeutic tools for avoiding or delaying the onset of illnesses brought on by oxidative stress. Formulation of silver Nanoparticles of *Duranta erecta* leaves extract may provide combined synergistic action which can be very useful in various systems to serve as a natural antioxidants.

5. Acknowledgements

I express my sincere gratitude to the Faculty of Pharmacy, Mandsaur, M. P., for providing the proper facilities to complete this work.

Declaration of interest: There is no conflict of interest.

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