RESEARCH PAPER

Phytochemical Screening and Evaluation of Antioxidant and Antibacterial Activity of Some Selected Medicinally Important Threatened Plants of Amroha District

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Abstract:

Medicinal plants constitute an important reservoir of bioactive compounds with potential antioxidant and antibacterial properties. The present study evaluated the phytochemical composition, antioxidant activity, and antibacterial potential of selected medicinal plants associated with the Amroha district of Uttar Pradesh. Eleven plant materials were extracted with methanol and subjected to qualitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical-scavenging assays. Antibacterial activity was assessed by the agar well diffusion method against *Salmonella*, *Enterococcus faecalis*, and *Pseudomonas syringae*. The extracts exhibited considerable variation in extraction yield and phytochemical profiles. Ashwagandha showed the highest TPC (91.75 ± 0.003 mg GAE/g extract), whereas Blue Pea Butterfly exhibited the highest TFC (0.693 ± 0.001 mg QE/g extract). Brahmi demonstrated the strongest antioxidant activity among the extracts (IC_{50} = 109.16 µg/ml), followed by Kalmegh and Guggul. Calendula showed the greatest antibacterial activity against *Salmonella* (8.0 mm), while Brahmi inhibited both *Salmonella* and *E. faecalis*. These findings demonstrate promising biological potential and support further phytochemical, pharmacological, and conservation-oriented investigation and sustainable utilization of these medicinal plant resources from the region in future.

Keywords: Medicinal plants, phytochemical screening, antioxidant activity, antibacterial activity

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1.0 Introduction

Medicinal plants have served as an essential source of healthcare and therapeutic agents since ancient times. Long before the development of modern synthetic medicines, human societies relied extensively on plants and plant-derived preparations for the prevention and treatment of diseases. Traditional systems of medicine, including Ayurveda, Unani, Siddha, and various indigenous healing practices, have documented the use of numerous plant species for conditions ranging from infectious diseases and inflammation to metabolic and chronic disorders. These traditional practices represent an important repository of ethnomedicinal knowledge and continue to provide valuable leads for the discovery of biologically active natural compounds [1, 2].

Ethnomedicine refers to the traditional knowledge, beliefs and practices associated with the use of natural resources, particularly plants, for maintaining health and treating diseases. The therapeutic properties of medicinal plants are largely attributed to the presence of diverse secondary metabolites, including phenolic compounds, flavonoids, alkaloids, tannins, terpenoids, glycosides, steroids, and essential oils. These constituents may exhibit a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and immunomodulatory effects. The scientific investigation of medicinal plants and their biologically active constituents has therefore contributed significantly to the development of phytotherapeutics, where standardized plant materials or plant-derived preparations are used for therapeutic purposes [3, 4].

Among the various biological properties of medicinal plants, antioxidant and antimicrobial activities have received considerable scientific attention. Oxidative stress, resulting from an imbalance between the production of reactive oxygen species and the body's antioxidant defense mechanisms, has been associated with cellular damage and the development of several chronic and degenerative disorders. Plant-derived phenolics, flavonoids, and other antioxidant compounds can neutralize or reduce the damaging effects of free radicals. Similarly, medicinal plants represent a potentially valuable source of antimicrobial agents because their secondary metabolites may inhibit the growth or survival of pathogenic microorganisms. In view of the increasing concern regarding antimicrobial resistance and the limitations associated with some conventional antimicrobial agents, the exploration of plant-based bioactive compounds has gained renewed importance [5, 6].

Despite their therapeutic and ecological significance, many medicinal plant species are increasingly threatened by biodiversity loss. Biodiversity loss has become a major environmental concern due to factors such as habitat destruction, urbanization, agricultural expansion, deforestation, climate-related changes, and unsustainable

harvesting practices. Medicinal plants are particularly vulnerable when naturally occurring populations are subjected to excessive or unregulated collection to meet commercial and traditional healthcare demands. Overexploitation of roots, stems, bark, gums, seeds, and whole plants may reduce natural regeneration and gradually threaten the long-term survival of valuable species. The loss of medicinal plant diversity not only affects ecosystems but may also result in the disappearance of traditional ethnomedicinal knowledge and potentially important sources of future therapeutic agents [7, 8].

The Amroha district of Uttar Pradesh possesses a diverse local flora that includes several medicinally important plants used traditionally for health and therapeutic purposes. Plant materials available or traditionally recognized in the region include Kalmegh (*Andrographis paniculata*), Brahmi Booti, Giloy (*Tinospora cordifolia*), Gond Chuniya, Shudh Guggul, Ashwagandha (*Withania somnifera*), Blue Pea Butterfly (*Clitoria ternatea*), Calendula, Nirgundi (*Vitex negundo*), Sat Mulethi, and Chirmati Safed. These plants and plant-derived materials represent a diverse group of botanical resources associated with traditional medicinal practices and may contain a variety of bioactive phytoconstituents. However, their local occurrence, botanical identity, abundance, conservation status, and biological activities may vary considerably and require systematic scientific documentation [9-13].

Several of these medicinal plants are known or traditionally valued for properties associated with the management of inflammation, infections, oxidative stress, weakness, metabolic disorders and other health conditions. For example, Kalmegh is widely recognized in traditional medicine for its bitter principles and therapeutic applications, whereas Giloy has been extensively used in traditional healthcare practices. Ashwagandha is valued for its restorative and adaptogenic properties, while *Clitoria ternatea*, Calendula, *Vitex negundo*, and other medicinal plants contain phytochemical constituents that may contribute to antioxidant or antimicrobial activity. Similarly, plant-derived products such as Guggul and other locally traded medicinal materials may contain important bioactive constituents. Nevertheless, the presence of traditional uses does not necessarily establish the magnitude, reproducibility, or comparative biological activity of plant materials obtained from a particular geographical region [14, 15].

Geographical location, climatic conditions, soil characteristics, seasonal variation, plant age, harvesting practices, and storage conditions can influence the phytochemical composition of medicinal plants. Consequently, plants growing in or collected from a specific region may demonstrate differences in the concentration and biological activity of their phytoconstituents compared with the same species obtained from other geographical locations. Therefore, the scientific evaluation of locally available medicinal flora is important for establishing evidence-based information regarding their therapeutic potential. Such investigations can also support the identification of promising plant species for further phytochemical characterization, standardization, conservation, and drug development [16, 17].

Although several medicinal plants have been investigated individually in different parts of India and elsewhere, comparatively limited scientific information is available regarding the antioxidant and antimicrobial potential of selected medicinal plants and plant materials associated with the local flora of Amroha district. Some traditionally used plants may be underexplored in terms of their regional occurrence, sustainable availability, conservation concerns, and experimentally validated biological properties. There is therefore a need to document and scientifically evaluate these valuable botanical resources before continued environmental pressure, habitat alteration, and overexploitation further affect their availability.

The present study designed to investigate selected medicinal plants associated with the local flora of Amroha district for their antioxidant and antimicrobial potential. The study aims to bridge traditional ethnomedicinal knowledge with scientific validation by generating experimental evidence regarding the biological activities of selected plant materials. Such an investigation may contribute to the identification of promising natural sources of antioxidant and antimicrobial agents while also highlighting the importance of documenting, conserving, and sustainably utilizing medicinal plant biodiversity. The findings may provide a scientific basis for further phytochemical investigation, isolation of bioactive constituents, development of phytotherapeutic preparations, and conservation-oriented research on the valuable medicinal flora of the Amroha region.

2.0 MATERIAL AND METHOD

2.1 Collection and Authentication of Plant

Eleven medicinal plant materials were selected for the present study and procured in dried powdered form. The selected materials included Kalmegh (*Andrographis paniculata*) whole herb/aerial parts powder, Brahmi Booti (*Bacopa monnieri*) leaf powder, Giloy (*Tinospora cordifolia*) stem powder, Gond Chuniya/Kamarkas plant gum or resin powder, Guggul Shudh (*Commiphora wightii*) purified gum resin powder, Ashwagandha (*Withania somnifera*) root powder, Blue Pea Butterfly (*Clitoria ternatea*) flower powder, Calendula (*Calendula officinalis*) flower-petal powder, Nirgundi (*Vitex negundo*) seed powder, Sat Mulethi (*Glycyrrhiza glabra*) licorice root extract powder, and Chirmati Safed (*Abrus precatorius*) seed powder. The plant

samples were authenticated by the Department of Botany, Vardhman College, Bijnor, Uttar Pradesh.

2.2 Preparation of Plant Extracts and Determination of Extraction Yield

The selected plant materials, namely Guggul, Mulethi, Nirgundi, Ashwagandha, Kalmegh, Giloy, Brahmi, Gondchuniya, Chirmati Safed, Bluepea Butterfly and Calendula, were processed separately and powdered. Methanolic extracts of the powdered plant materials were prepared under identical extraction conditions. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extracts were weighed and stored at -20°C until further analysis [18].

The percentage extraction yield was calculated using the following equation:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of powdered plant material (g)}} \times 100$$

Where the weight of dried extract represents the mass of the concentrated extract obtained after solvent removal and the weight of powdered plant material represents the initial mass of the plant material used for extraction.

2.3 Phytochemical Screening

The methanolic plant extracts were subjected to preliminary phytochemical screening to determine the presence of major classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins and steroids. The development of characteristic colour changes, precipitates, or persistent foam was considered indicative of the respective phytochemical constituents [19].

2.3.1 Test for Flavonoids

An aliquot of the crude extract was treated with 5 ml of dilute ammonia solution followed by the addition of concentrated sulfuric acid. The development of a yellow colour, which disappeared upon standing, indicated the presence of flavonoids.

2.3.2 Test for Alkaloids

The crude extract was treated with Wagner's reagent. The formation of a reddish-brown precipitate indicated the presence of alkaloids. Mayer's and Dragendorff's tests were also employed for preliminary confirmation of alkaloids, with characteristic precipitate formation considered a positive reaction.

2.3.3 Test for Phenolic Compounds and Tannins

For the detection of phenolic compounds and tannins, 1 ml of the extract was treated with 1 ml of 0.008 M potassium ferricyanide, followed by 1 ml of 0.02 M ferric chloride containing 0.1 N hydrochloric acid. The development of a blue-black colour indicated the presence of phenolic compounds and tannins.

2.3.4 Test for Saponins

The crude extract was mixed with 5 ml of distilled water and shaken vigorously. The formation of persistent foam was considered indicative of the presence of saponins.

2.3.5 Test for Steroids

Approximately 0.5 ml of the crude extract was treated with 2 ml of acetic anhydride followed by 2 ml of concentrated sulfuric acid. A characteristic colour changes from violet toward blue or green indicated the presence of steroidal constituents.

2.3.6 Test for Glycosides

The extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution, followed by careful addition of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of glycosides, particularly cardenolide-type glycosides.

2.4 Determination of Total Phenolic Content

The total phenolic content (TPC) of the methanolic plant extracts was determined using the Folin-Ciocalteu colorimetric method, with gallic acid as the reference standard. A stock solution of each plant extract was prepared at a concentration of 1 mg/ml. 50 µl of the extract solution was mixed with 450 µl of distilled water, followed by addition of 500 µl of Folin-Ciocalteu reagent and 2.0 ml of 20% sodium carbonate solution. The reaction mixture was thoroughly mixed and incubated at room temperature to allow colour development. The absorbance was subsequently measured at 765 nm using a UV–visible spectrophotometer [20].

A calibration curve was prepared using different concentrations of gallic acid under identical experimental conditions. The total phenolic content of each extract was calculated from the corresponding gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract). The calibration curve obtained for gallic acid showed a regression equation of $y=11.98x-0.0779$ with $R^2=0.9805$. All measurements were performed in triplicate and the results were expressed as mean ± standard deviation.

2.5 Determination of Total Flavonoid Content

The total flavonoid content (TFC) of the methanolic plant extracts was determined using the aluminium chloride ($AlCl_3$) colorimetric method, with quercetin as the reference standard. Different concentrations of quercetin were used to construct the calibration curve. The plant extract solutions and quercetin standards were treated with the appropriate reagents used for the aluminium chloride colorimetric reaction and incubated at room temperature for colour development. The absorbance was measured at 415 nm using a UV-visible spectrophotometer. The total flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract). The calibration curve showed a good linear relationship between quercetin

concentration and absorbance. All measurements were performed in triplicate and the results were expressed as mean ± standard deviation [21].

2.6 Evaluation of Antioxidant Activity Using DPPH Radical-Scavenging Assay

The antioxidant activity of the plant extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. A fresh 0.1 mM DPPH solution was prepared in methanol and protected from light. The plant extracts were dissolved in methanol and prepared at concentrations of 25-400 µg/ml. Ascorbic acid was used as reference standard and was prepared at same concentrations. For each concentration, an appropriate volume of extract and ascorbic acid solution was mixed with DPPH working solution to obtain required final reaction volume. The reaction mixtures were thoroughly mixed and incubated at room temperature in the dark for 30 min. A DPPH solution containing methanol without extract was used as the control. After incubation, the decrease in absorbance resulting from reduction of DPPH radical was measured at 517 nm using a UV-visible spectrophotometer. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation (SD). IC50 values were determined from concentration response curves, with lower IC_{50} values indicating greater antioxidant activity [22]. The percentage of DPPH radical-scavenging activity was calculated using formula:

$$\% \text{ DPPH Scavenging Activity} = \frac{A1 - A2}{A1} \times 100$$

Where A1 is absorbance of DPPH control and A2 is absorbance of reaction mixture containing test extract or ascorbic acid.

2.7 Evaluation of Antibacterial Activity Using Agar Well Diffusion Method

The antibacterial activity of the selected plant extracts was evaluated by the agar well diffusion method. The aqueous plant extracts were tested against *Salmonella*, *Enterococcus faecalis*, and *Pseudomonas syringae*. For the assay, bacterial cultures were uniformly swabbed onto the agar plates, and wells of approximately 5 mm diameter were prepared aseptically using a sterile well cutter. 100 µl of each plant extract was added to the respective wells using a micropipette. The plant extracts were tested at a concentration of 200 µg/ml. Rifampicin (50 µg/ml) was used as the reference antibacterial agent for all tested bacterial strains. The inoculated plates were incubated at 37 °C for 24 h. After incubation, the diameter of the clear zone surrounding each well was measured and recorded in millimetres (mm) [23].

3.0 RESULT & DISCUSSION

3.1 Extraction Yield of Plant Extracts

The methanolic extracts of plants were prepared under similar extraction conditions. The extraction yield varied considerably among the investigated plant materials (table 3.1). Guggul, Mulethi, Kalmegh, and Giloy exhibited comparatively higher extraction yields, ranging from 45% to 50%. Bluepea Butterfly showed extraction yields in the range of 40-45%, whereas Nirgundi, Ashwagandha, Brahmi, Gondchuniya, Chirmati Safed, and Calendula showed yields between 30% and 40%.

Table 3.1 Percentage Yield of Methanolic Plant Extracts

Plant Name	Final Dry Weight (g)	Extraction Yield (%)
Guggul	2.3	46
Mulethi	2.4	48
Nirgundi	1.9	38
Ashwagandha	1.9	38
Kalmegh	2.3	46
Giloy	2.3	46
Brahmi	1.8	36
Gondchuniya	1.9	38
Chirmati Safed	1.8	36
Bluepea Butterfly	2.2	44
Calendula	1.6	32

3.2 Phytochemical Screening

Preliminary phytochemical screening (table 3.2) revealed the presence of several classes of secondary metabolites in the investigated methanolic plant extracts. Alkaloids, flavonoids, glycosides, phenolic compounds, tannins, saponins and steroids were detected in varying patterns among the plant extracts.

Table 3.2 Phytochemical Profile of Methanolic Plant Extracts

Plant Extract	Alka-loid	Flavonoid	Phenolic compound	Tannin	Glycoside	Saponin	Steroid
Guggul	−	+	−	+	++	+	−
Mulethi	−	++	−	+	+	+	−
Nirgundi	+++	−	++	++	−	+++	++
Ashwagandha	+	++	+	+	+	−	−
Kalmegh	−	+	−	+	+	+	−
Giloy	+	+	+	−	++	+++	−

Brahmi	+++	+	++	++	+	+++	++
Gondchuniya	−	+	+	−	−	+	−
Chirmati Safed	+	−	+	−	+	−	++
Bluepea Butterfly	+	+	+	+	+	+	++
Calendula	+	+	+	+	+	+	+

Note: *Absent (−), Weakly Positive (+), Moderately Positive (++), Strongly Positive (+++)*

Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, and steroids in varying combinations among the methanolic plant extracts. Nirgundi and Brahmi showed comparatively strong reactions for several phytoconstituents, while Mulethi and Ashwagandha showed notable flavonoid reactions. The results demonstrate considerable phytochemical diversity among the investigated plant extracts.

3.3 Total Phenolic Content

The total phenolic content (TPC) of the methanolic plant extracts was determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE) and result is represented in table 3.3. The calibration curve for gallic acid showed good linearity, with the regression equation y = 11.98x-0.0779 and a coefficient of determination (R^2 = 0.9805).

Table 3.3 Total Phenolic Content of Methanolic Plant Extracts

Plant Extract	Mean Absorbance at 765 nm	TPC (mg GAE/g extract)
Guggul	0.092	5.71 ± 0.031
Mulethi	0.311	13.05 ± 0.005
Nirgundi	0.031	3.60 ± 0.002
Ashwagandha	2.672	91.75 ± 0.003
Kalmegh	0.392	15.62 ± 0.004
Giloy	0.088	5.53 ± 0.031
Brahmi	0.122	6.64 ± 0.005
Gondchuniya	0.448	17.59 ± 0.002
Chirmati Safed	0.088	5.51 ± 0.003
Bluepea Butterfly	0.123	6.64 ± 0.005
Calendula	0.468	18.19 ± 0.002

The total phenolic content varied substantially among the investigated plant extracts, ranging from approximately 3.34 to 91.75 mg GAE/g extract. Ashwagandha exhibited the highest phenolic content (91.75 ± 0.003 mg GAE/g extract), indicating a comparatively high abundance of Folin-Ciocalteu-reactive phenolic constituents. Calendula (18.19 ± 0.002 mg GAE/g), Gondchuniya (17.59 ± 0.002 mg GAE/g), and Kalmegh (15.62 ± 0.004 mg GAE/g) also exhibited comparatively high phenolic contents. Mulethi showed a TPC of approximately 13.05 mg GAE/g, whereas Brahmi and Bluepea Butterfly showed values of approximately 6.64± 0.005 mg GAE/g. Lower phenolic contents were observed for Guggul, Giloy, Chirmati Safed, Nirgundi.

3.4 Total Flavonoid Content

The total flavonoid content (TFC) of methanolic plant extracts was determined by the aluminium chloride colorimetric method using quercetin as reference standard. The quercetin calibration curve exhibited good linearity, with the regression equation y = 9.2161x-0.1468 and R^2 = 0.9877.

Table 3.4 Total Flavonoid Content of Methanolic Plant Extracts

Plant extract	Mean absorbance at 415 nm	TFC (mg QE/g extract)
Guggul	0.094	0.092 ± 0.0012
Mulethi	0.476	0.236 ± 0.0016
Nirgundi	0.068	0.081 ± 0.0004
Ashwagandha	0.917	0.404 ± 0.0004
Kalmegh	0.412	0.212 ± 0.0004
Giloy	0.516	0.252 ± 0.0004
Brahmi	0.235	0.145 ± 0.0016
Gondchuniya	1.212	0.516 ± 0.0004
Chirmati Safed	0.598	0.283 ± 0.0004
Bluepea Butterfly	1.678	0.693 ± 0.001
Calendula	0.656	0.305 ± 0.0004

The total flavonoid content of the investigated methanolic extracts ranged from 0.081 to 0.693 mg QE/g extract. Bluepea Butterfly exhibited the highest flavonoid content (0.693 ± 0.001 mg QE/g extract), followed by Gondchuniya (0.516 ± 0.0004 mg QE/g), Ashwagandha (0.404 ± 0.0004 mg QE/g)

and Calendula (0.305 ± 0.0004 mg QE/g). Chirmati Safed, Giloy, Mulethi, and Kalmegh showed intermediate flavonoid contents of approximately 0.283, 0.252, 0.236, and 0.212 mg QE/g extract, respectively. Brahmi, Guggul and Nirgundi exhibited comparatively lower flavonoid contents. The lowest TFC was observed in Nirgundi (0.081 ± 0.0004 mg QE/g extract).

3.5 Evaluation of Antioxidant Activity of Plant Extracts

All plant extracts demonstrated concentration dependent antioxidant activity in the 25-400 µg/ml range, according to DPPH assay. The % inhibition increased with concentration for all compounds, demonstrating effective free radical scavenging ability. The results were represented in table (3.5-3.17) and figure (3.1-3.14).

Table 3.5 Radical Scavenging Activity and IC50 of Ascorbic Acid (Standard Drug)

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	45.31 ± 0.16	46.41 µg/ml
50	52.45 ± 0.24	
100	59.01 ± 0.23	
150	63.54 ± 0.21	
200	69.17 ± 0.24	
250	75.73 ± 0.27	
300	81.20 ± 0.24	
350	90.42 ± 0.22	
400	98.28 ± 0.16	

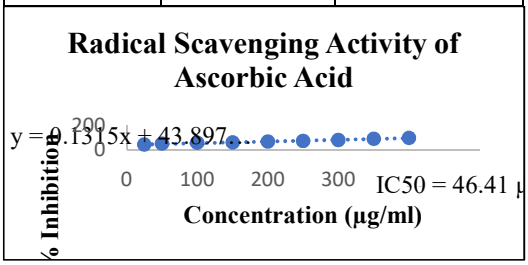

Figure 3.1 %Inhibition of Ascorbic Acid (Standard Drug) with IC50 Value

Table 3.6 Radical Scavenging Activity and IC50 of Kalmegh

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	29.09 ± 0.63	124.23 µg/ml
50	37.86 ± 0.55	
100	46.01 ± 0.63	
150	58.30 ± 0.70	
200	65.25 ± 0.63	
250	74.27 ± 0.86	
300	77.34 ± 0.63	
350	82.22 ± 0.65	
400	88.94 ± 0.61	

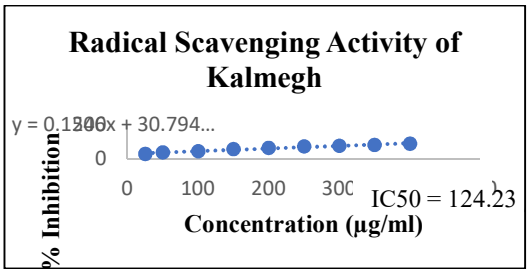

Figure 3.2 %Inhibition of Kalmegh with IC50 Value

Table 3.7 Radical Scavenging Activity and IC50 of Brahmi

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	32.09 ± 0.47	109.16 µg/ml
50	40.93 ± 0.55	
100	49.13 ± 0.71	
150	61.10 ± 0.78	
200	67.05 ± 0.70	
250	72.06 ± 0.63	
300	77.03 ± 0.70	
350	83.27 ± 0.71	
400	89.15 ± 0.73	

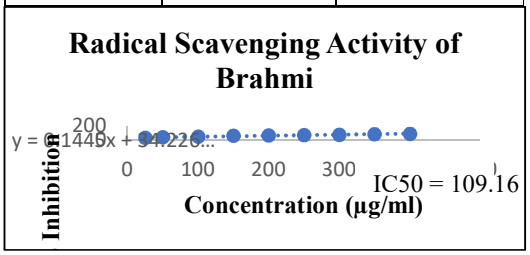

Figure 3.3 %Inhibition of Brahmi with IC50 Value

Table 3.8 Radical Scavenging Activity and IC50 of Giloy

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	10.94 ± 0.63	295.17 µg/ml
50	18.91 ± 0.63	
100	25.02 ± 0.86	
150	33.65 ± 0.86	
200	38.13 ± 0.94	
250	46.02 ± 0.86	
300	49.15 ± 0.76	
350	55.27 ± 0.82	
400	63.93 ± 0.87	

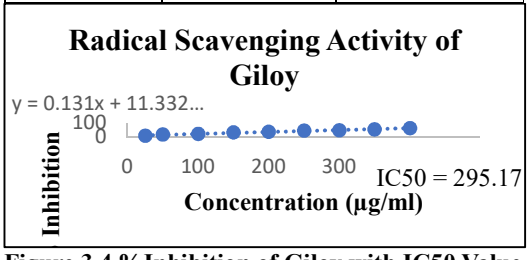

Figure 3.4 %Inhibition of Giloy with IC50 Value

Table 3.9 Radical Scavenging Activity and IC50 of Gondchuniya

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	17.08 ± 0.86	255.46 µg/ml
50	23.13 ± 0.79	
100	28.28 ± 0.94	
150	35.00 ± 0.78	
200	41.90 ± 0.86	
250	46.90 ± 0.82	
300	58.75 ± 0.94	
350	63.51 ± 0.86	
400	69.57 ± 0.91	

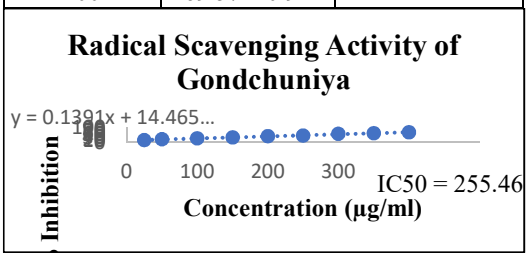

Figure 3.6 %Inhibition of Gondchuniya with IC50 Value

Table 3.10 Radical Scavenging Activity and IC50 of Guggul Shudh

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	27.97 ± 0.61	131.61 µg/ml
50	39.01 ± 0.63	
100	48.16 ± 0.62	
150	57.24 ± 0.71	
200	63.08 ± 0.70	
250	67.15 ± 0.71	
300	72.38 ± 0.79	
350	78.77 ± 0.86	
400	85.75 ± 0.94	

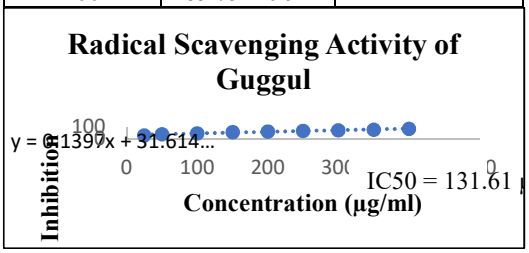

Figure 3.7 %Inhibition of Guggul with IC50 Value

Table 3.11 Radical Scavenging Activity and IC50 of Ashwagandha

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	14.22 ± 0.94	230.04 µg/ml
50	24.69 ± 0.94	
100	35.26 ± 0.86	
150	41.77 ± 0.81	
200	46.41 ± 0.78	
250	50.83 ± 0.70	
300	58.38 ± 0.79	
350	67.40 ± 0.71	
400	75.16 ± 0.78	

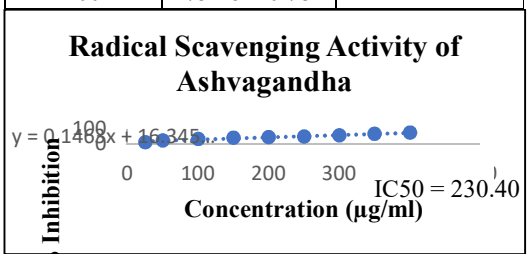

Figure 3.8 %Inhibition of Ashwagandha with IC50 Value

Table 3.12 Radical Scavenging Activity and IC50 of Blue Pea Butterfly

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	26.61 ± 0.79	145.95 µg/ml
50	37.08 ± 1.02	
100	48.02 ± 1.02	
150	54.95 ± 0.91	
200	60.01 ± 0.94	
250	63.96 ± 1.02	
300	68.19 ± 0.94	
350	77.16 ± 0.78	
400	83.50 ± 0.92	

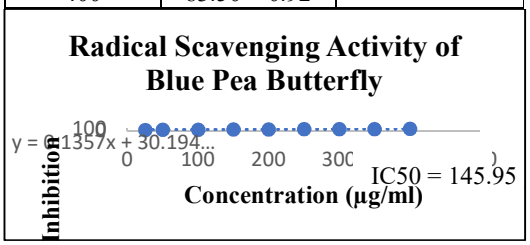

Figure 3.9 %Inhibition of Blue Pea Butterfly with IC50 Value

Table 3.13 Radical Scavenging Activity and IC50 of Calendula

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	23.94 ± 0.87	202.68 µg/ml
50	31.98 ± 0.78	
100	37.81 ± 0.71	
150	43.79 ± 0.79	
200	48.94 ± 0.71	
250	56.05 ± 0.86	
300	63.18 ± 0.86	
350	68.35 ± 0.78	
400	76.11 ± 0.70	

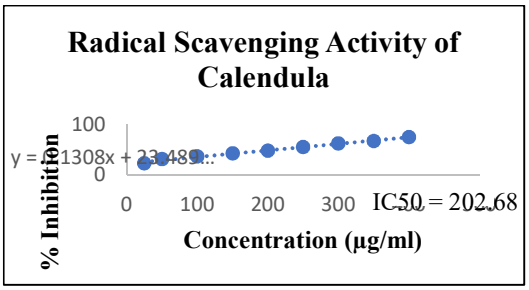

Figure 3.10 %Inhibition of Calendula with IC50 Value

Table 3.14 Radical Scavenging Activity and IC50 of Nirgundi

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	10.78 ± 0.47	302.68 µg/ml
50	18.23 ± 0.55	
100	26.98 ± 0.51	
150	33.70 ± 0.55	
200	39.53 ± 0.63	
250	45.53 ± 0.59	
300	49.59 ± 0.62	
350	55.09 ± 0.63	
400	59.22 ± 0.60	

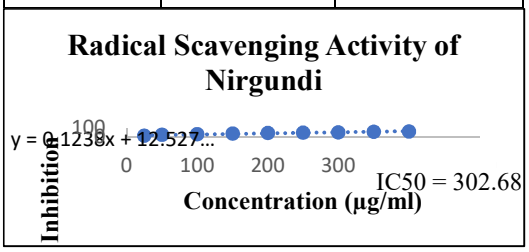

Figure 3.11 %Inhibition of Nirgundi with IC50 Value

Table 3.15 Radical Scavenging Activity and IC50 of Mulethi

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	23.41 ± 0.47	207.48 µg/ml
50	29.09 ± 0.55	
100	36.28 ± 0.63	
150	41.33 ± 0.55	
200	49.03 ± 0.47	
250	57.84 ± 0.41	
300	62.18 ± 0.42	
350	69.96 ± 0.40	
400	75.05 ± 0.55	

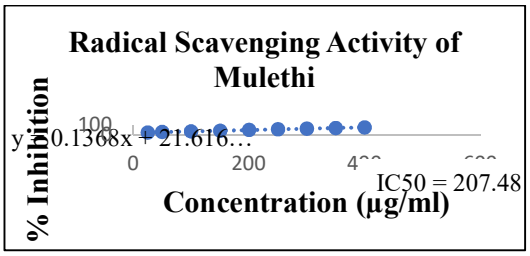

Figure 3.12 %Inhibition of Mulethi with IC50 Value

Table 3.16 Radical Scavenging Activity and IC50 of Chirmati Safed

Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC50
25	22.71 ± 0.86	171.82 µg/ml
50	33.39 ± 0.79	
100	41.79 ± 0.79	
150	48.08 ± 0.82	
200	57.66 ± 0.86	
250	61.77 ± 0.84	
300	69.05 ± 0.81	
350	74.02 ± 0.80	
400	82.47 ± 0.78	

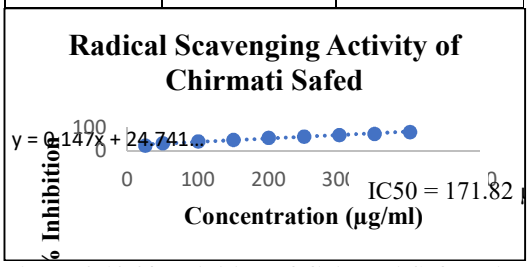

Figure 3.13 %Inhibition of Chirmati Safed with IC50 Value

Table 3.17 Comparative Radical Scavenging Activity (%Inhibition) of Ascorbic Acid (Standard Drug) and Plant Extracts

Plant Extract	Concentartion (µg/ml)								
	25	50	100	150	200	250	300	350	400
Ascorbic Acid	45.31	52.45	59.01	63.54	69.17	75.73	81.20	90.42	98.28
Mulethi	23.41	29.09	36.28	41.33	49.03	57.84	62.18	69.96	75.05
Kalmegh	29.	37.	46.	58.	65.	74.	77.	82.	88.

	09	86	01	30	25	27	34	22	94
Nirgunda	10.78	18.23	26.98	33.70	39.53	45.53	49.59	55.09	59.22
Guggul	27.97	39.01	48.16	57.24	63.08	67.15	72.38	78.77	85.75
Chirmati Safed	22.71	33.39	41.79	48.08	57.66	61.77	69.05	74.02	82.47
Chalendula	23.94	31.98	37.81	43.79	48.94	56.05	63.18	68.35	76.11
Blue pea Butterfly	26.61	37.08	48.02	54.95	60.01	63.96	68.19	77.16	83.50
Gondchuniya	17.08	23.13	28.28	35.00	41.90	46.90	58.75	63.51	69.57
Ashwagandha	14.22	24.69	35.26	41.77	46.41	50.83	58.38	67.40	75.16
Brahmi	32.09	40.93	49.13	61.10	67.05	72.06	77.03	83.27	89.15
Giloy	10.94	18.91	25.02	33.65	38.13	46.02	49.15	55.27	63.93

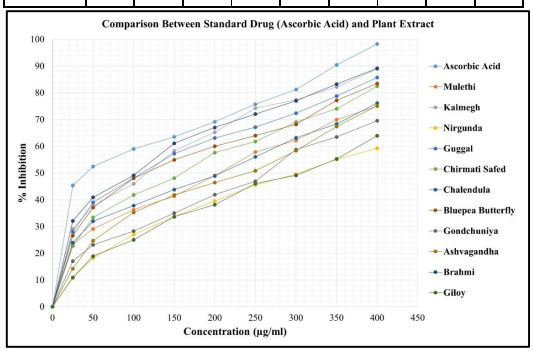

Figure 3.14 Comparison Between Standard Drug (Ascorbic Acid) and Plant Extract

The DPPH assay showed a concentration-dependent increase in radical-scavenging activity in all plant extracts. Among the extracts, Brahmi showed the highest antioxidant activity (IC50= 109.16 µg/ml), followed by Kalmegh (124.23 µg/ml) and Guggul Shudh (131.61 µg/ml). Giloy and Nirgundi showed the lowest activity, with IC50 values of approximately 295.17 and 302.68 µg/ml, respectively. Ascorbic acid exhibited strongest activity (IC50=46.41 µg/ml), confirming its superior radical-scavenging capacity compared with the plant extracts.

3.6 Evaluation of Antibacterial Activity of Plant Extracts

The antibacterial activity of the aqueous plant extracts was evaluated by the agar well diffusion method against *Salmonella*, *Enterococcus faecalis*, and *Pseudomonas syringae*. The extracts exhibited variable antibacterial activity depending on the bacterial strain. The results were displayed in table 3.18 and figure (3.15-3.17).

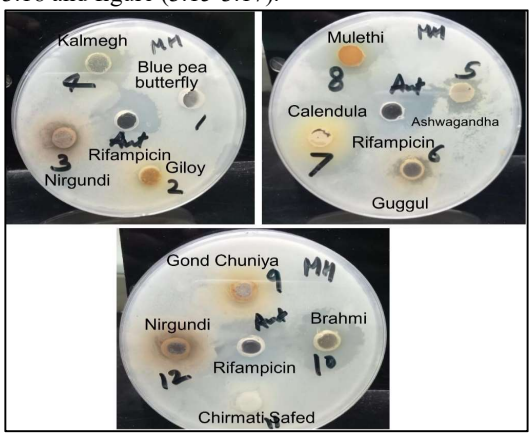

Figure 3.15 Zone of Inhibition of Plant Extract and Standard Drug Against *Salmonella*

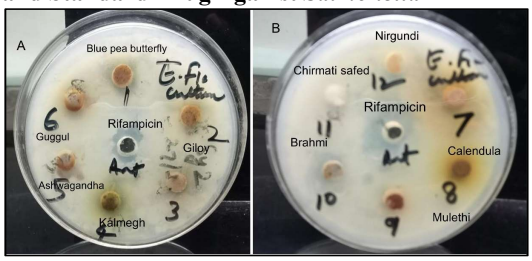

Figure 3.16 Zone of Inhibition of Plant Extract and Standard Drug Against *Enterococcus faecalis*

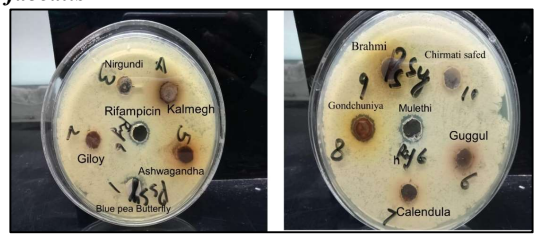

Figure 3.17 Zone of Inhibition of Plant Extract and Standard Drug Against *Pseudomonas syringae*

Table 3.18 Comparative Antibacterial Activity of Rifampicin and Plant Extracts by Agar Well Diffusion Assay

Plant extract	Zone of Inhibition (mm)		
	Salmonella (Gram -)	***E. faecalis*** (Gram +)	***P. syringae*** (Gram -)
Blue pea Butterfly	-	3.0 mm	-
Giloy	-	-	-
Nirgundi Seeds	-	-	-
Kalmegh	5.0 mm	-	-
Ashwagandha	4.0 mm	-	-
Guggul	2.0 mm	-	-
Calendula	8.0 mm	-	-
Mulethi	7.0 mm	-	1.0 mm
Gondchuniya	-	-	-
Brahmi	7.0 mm	5.0 mm	-
Chirmati Safed	-	-	-
Rifampicin (Standard Drug)	8.67 mm	6.5 mm	1.5 mm

The antibacterial activity of the plant extracts was found to be strain dependent, with *Salmonella* showing greatest susceptibility. Among the plant extracts, Calendula showed highest inhibition zone (8.0 mm), followed by Mulethi and Brahmi (7.0 mm each), while Kalmegh, Ashwagandha and Guggal produced inhibition zones of 5.0, 4.0 and 2.0 mm, respectively. Against *Salmonella*, rifampicin produced inhibition zones of 8.67 mm, indicating that Calendula showed an inhibition zone comparable to reference drug. Against *E. faecalis*, Brahmi (5.0 mm) and Blue Pea Butterfly (3.0 mm) exhibited detectable inhibition, whereas rifampicin (50 µg/ml) produced zones of 6.5 mm). Against *P. syringae*, Mulethi (1.0 mm) was the only plant extract showing detectable inhibition, while rifampicin produced zones of 1.5 mm.

3.7 Discussion

The present study demonstrated considerable variation in the phytochemical composition, antioxidant potential, and antibacterial activity of selected medicinal plants associated with the Amroha district. The methanolic extraction yields varied from 32% in Calendula to 48% in Mulethi, indicating differences in the extractable constituents among plant materials. Preliminary phytochemical screening further confirmed substantial chemical diversity, with alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, and steroids detected in different combinations. Nirgundi and Brahmi showed strong reactions for several phytochemical groups, supporting their potential as important sources of bioactive constituents.

The quantitative phytochemical results revealed marked differences in total phenolic and flavonoid contents. Ashwagandha exhibited the highest TPC (91.75 ± 0.003 mg GAE/g), whereas Blue Pea Butterfly showed the highest TFC (0.693 ± 0.001 mg QE/g). However, antioxidant activity did not correspond directly with either TPC or TFC. Brahmi showed the strongest antioxidant activity among the extracts (IC_{50} = 109.16 µg/ml), followed by Kalmegh (124.23 µg/ml) and Guggul (131.61 µg/ml), whereas Nirgundi and Giloy demonstrated comparatively weaker activity. Ascorbic acid remained substantially more active, with an IC_{50} of 46.41 µg/ml. This variation suggests that antioxidant activity may depend not only on total phenolic or flavonoid content but also on the specific chemical composition, structural characteristics, and possible synergistic interactions among multiple phytoconstituents.

The antibacterial findings also demonstrated pronounced plant- and strain-dependent activity. Calendula produced the largest inhibition zone against *Salmonella* (8.0 mm), closely approaching rifampicin (8.67 mm), while Brahmi exhibited activity against both *Salmonella* and *E. faecalis*. Mulethi showed limited activity against *P. syringae*. Overall, these findings provide experimental support for the biological potential of selected medicinal plants from Amroha and emphasize the importance of further phytochemical characterization, isolation of active constituents, standardization, and conservation-oriented research.

Conclusion

The present study provides evidence of antioxidant and antibacterial potential of selected medicinal plants associated with the Amroha district and highlights their significance as valuable sources of biologically active phytoconstituents. The investigated plants exhibited considerable variation in extraction yield and phytochemical composition, with alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, and steroids detected in different combinations. Quantitative analysis further demonstrated substantial differences in total phenolic and flavonoid contents among the extracts. All tested extracts demonstrated concentration-dependent DPPH radical-scavenging activity. Brahmi exhibited the strongest antioxidant activity among the plant extracts, followed by Kalmegh and Guggul, whereas Giloy and Nirgundi showed

comparatively weaker activity. Ascorbic acid demonstrated superior antioxidant activity compared with all plant extracts. The antibacterial evaluation revealed strain-dependent activity, with Calendula showing the highest inhibition against *Salmonella*, while Brahmi demonstrated activity against both *Salmonella* and *Enterococcus faecalis*. The findings support the therapeutic potential of selected medicinal plants of Amroha district. Further studies involving detailed phytochemical profiling, isolation and characterization of active constituents, mechanistic investigations, toxicity evaluation and in-vivo validation are warranted. Importantly, systematic documentation and sustainable utilization of these medicinal plant resources may contribute simultaneously to natural-product drug discovery and conservation of valuable plant biodiversity.

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