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Antimicrobial and antioxidant efficacy from selected medicinal plants

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Abstract
The indiscriminate application of antimicrobial compounds has gained lot of attention wjich are able to cause global health issues, which is outcome of bacterial resistance. The present research focused on the antimicrobial and free radical efficacies of polar extracts of the two medicinal plants *Derris elliptica and Ailanthus excels* on various clinical isolates. Both plants were sequentially portioned in different solvents, at different dose level. The free radical scavenging was studied using the DPPH assay. In *D. elliptica* against *E. coli* we found that both chloroform and methanolic extracts of bark showed better activity (IZ-14mm) as compared to leaves. Against *S. aureus* the observations were in contrast to earlier as leaves had better efficacy (IZ -14mm) as compared to bark. Against *P. aeruginosa* highest potency found in methanolic fraction of leaves (IZ -16mm) while against *B. subtilis* partial efficacy was found in some cases. In fungal efficacy when extracts were tested against *C. albicans* it was observed that maximum efficacy was in methanolic extracts of leaves (IZ-12mm) while in chloroform extracts activity was at par both in leaves and bark. Against *P. chrysogenum* maximum efficacy found in chloroform fraction of leaves (IZ-14mm) while against *A. niger* resistivity was found in both parts. Against *F. moniliforme* maximum efficacy found in chloroform fraction of bark (IZ-16 mm). In *A. excelsa* when extracts were tested against different bacterial strains were studied activity was lower as compared to *D. elliptica*
It was observed that against *E. coli* methanolic extracts of leaves and bark have at par activity (IZ-12 mm) while chloroform extract of bark did not show any activity. Against *S. aureus* maximum activity (IZ-16 mm) was observed in chloroform extracts of leaves while no activity was observed in methanolic extracts.
Against *P. aeruginosa* no activity was observed in any sample so it was found to be resistant while in *B. subtilis* maximum efficacy (IZ-18mm) was observed in methanolic extracts of bark.
In case of fungal efficacy, it was observed that against *C. albicans* both methanolic and chloroform extract of leaves had potent activity while in bark no efficacy was observed. Maximum activity was observed in chloroform extract of leaves (IZ-10mm). Against *P. chrysogenum* except chloroform extracts of leaves no activity was observed in any sample thus it was found to be resistant. Further against *A. niger* moderate efficacy was observed in both chloroform and methanolic extracts of leaves (IZ-8 and 6mm). Against *F. moniliforme* at par efficacy was observed in chloroform extracts of leaves and bark (IZ-12mm each).
Both plants exhibited antioxidant activities by various assys like DPPH, FRAP, catalase and Peroxidase. The plant extracts exhibited antimicrobial and free radical scavenging efficacies and were having rich source of natural products of active constituents used as traditional medicine

Keywords: Antimicrobial activity, antioxidant activity, medicinal plants, Derris Elliptica, Ailanthus Excelsa, dpph assay, free radical scavenging

Introduction
In recent years a lot of attention has been attracted towards bioactive compounds isolated from plants that possess a variety of medicinal and biomedical efficacies. These compounds act as additional nutritional components found in minute amount, and have distinctiveness globally in chemical texture including application and classified as flavonoids, sterols, flavones, phenols and alkaloids etc. As per their role and accumulation these are categorized as divided into primary and secondary metabolites. Now it has been proved that these bioactive compounds are key component of the plant cell metabolism which also possess specific proteins and cells. There is narrow gap between primary and bioactive compounds as they are connected and plays significant role in various biological routes (Dwivedi and Dwivedi, 2007) [7] Attention has been diverted in natural products based on plants as a resustaining medicinal and health ailments due to cost effective treatment of chemically synthesized drugs along with their toxicities in the sustainability of individual medical aid and development, and the exploitation of new plant-mediated drugs. Some reports on recent investigations and economic expenses, medicinal plants will, ultimately, continue to have crucial role in developing as a health aid.
Several drastic physiological phenomena like obesity, cancer, cardiovascular disease, diabetes and neurological disorders, caused by oxidative stress (Vincent *et al*., 2007) [28]. Further, the higher amount of free radical results in disruption of oxidant and free radical scavenging efficacy, which is root cause of generation of free radicals (Ardeshirlarijani *et al*., 2019) [3]. Frequently, these ROS are synthesized at minute quantity, but when infected occurs, rate of free radicals increases and results in causing mortality to cells. Antioxidant defense process occurs in all living cells to remove the drastic impacts of ROS. Antioxidants are compounds which initiate electrons to disrupted cells to resist and steady drastic effects of ROS. They change ROS into garbage molecules which body secrets (Rahman *et al*., 2015) [21].
Catalase (CAT) and glutathione peroxidases (GPX) belongs to class of enzymatic antioxidants. Micronutrients (metals and vitamins) are generally non -enzymatic class. As we all

know ascorbic acid is polar vitamin; vitamin E is non-polar. Other molecules, like albumin, possess tremendous antioxidant efficacy, including Quercetin (Serban *et al*., 2016) [23] Rebamipide, and Trolox (Hawash *et al*., 2020) [9], generally recommended as potent antioxidant compounds.

Since many decades, plants have recommended for therapeutic ventures, including the combating of infectious diseases (Kuruppu *et al*., 2019) [14]. Aromatic plants and spices have crucial role for the pharmaceutical industry, cosmetics, and food. Synthetic drugs have been broadly applied on microorganisms; unfortunately, they develop resistance to many antibiotics as a result of their arbitrary use. Furthermore, these antibiotics sometimes results in allergic reactions and immune restraint. The consumption of plants is non-toxic for human health and for environment to fight against these microbes.

Thus, these bioactive compounds based on medicinal plants has crucial role in health care, and play twin role in innovation of such drugs as they are backbone for designing such medicine, a natural blue print for the designing of novel molecules or; natural product engaged in combating many diseases. Therefore, these medicinal plants have been attracted for exploitation of isolation of such compounds to impart knowledge about their underlying mode of action with reduced toxicity and with cost effective features (Mickle, 2001) [17]. The enhanced risk and toxicities of chemotherapeutics and occurrence of multiple drug resistance mutants in pathogenic bacteria forced us to divert towards search of such novel drugs having antimicrobial potential.

Derris elliptica belongs to leguminous family from Southeast Asia including Pacific islands. The roots of *D. elliptica* possess rotenone, a robust insecticide and fish poison present in roots, (Fryer *et al*., 1923) it was earlier recommended as a natural insecticide to fight against crop pests like peas. Inspite of all this Derris is used as a food plant by *Lepidopteran* larvae including *Batrachedra amydraula.* There are studies on biocompatibilities of this plant. Jessa *et al* (2015) [12] reported the efficacy of dehydrated extract of *Derris elliptica* stem are responsible for some enzymatic variations in the plasma of *Clarias gariepinus.* However, Paul *et al* (2019) [20] reported study on therapeutic screening of *Derris robusta.*

Ailanthus excelsa Roxb. (Simaroubaceae) is identified by its *Mahanimba* as it has similar features *Azadirachita indica* and *Maharukha* as it appears big structure. *Ailanthus* word is from ailanto meaning flora of ecstasy and also by other names in Indian local languages (Database, 2000). The alcoholic extracts of different plant parts at concentration of 255mg/kg b.wt. showed are markable anti scion and early antifertility efficacy in female albino mice (Lavhale and Mishra, 2007) [15]. The polar and non-polar fractions (Quassinoid fraction) of bark was screened was against *Entamoeba histolytica* having anti-amoebic properties taking metronidazole as market medicine. The EC coefficient for these extracts was found to be in range of were 150-200 µg mL/g (Yoganandam *et al*., 2009a) [29].

Material and Method

Harvesting of Test samples

The Plant material of *Derris elliptica* were harvested from the outskirts of Jamwaramgarh from Jaipur district. The plant of authenticated from Rajasthan University, Jaipur, India. (Ref. RU/2019/532). The different plant parts (bark and leaves were shade dried initially.

Different Plant parts of *Ailanthus* ***excels*** (Bark and leaves) were harvested from Jaipur, University of Rajasthan. Further it was authenticated from Rajasthan University, Jaipur, India. (Ref. RU/2019/841). The plant materials were cleaned instantly with running tap water, and moisture was removed in closed room and were made to powder through grinder.

Antimicrobial Activity

Antimicrobial efficacy of isolated sequential extracts has been carried out against various clinical important strains. Various microbial strains have been collected for pursuing the activity

Microorganisms Used

Clinically harvested and cultured microbes of *E. coli* (MTCC No.1687), *Staphylococcus aureus* (MTCC No. 737), *B. subtilis* (MTCC No. 441) and *Pseudomonas aeruginosa* (MTCC No. 424) and fungal isolates *viz. Aspergillus flavus* (ATCC No. 56766), *Fusarium oxysporium* (ATCC No.48112), *Aspergillus niger* (ATCC No. 16888) and *Candida albicans* (ATCC No 9178.) were isolated and subcultured from the mother colonies provided by Microbiology Laboratory, SMS Medical College, Jaipur, India.

Antibacterial Assay

This assay of the various extracts (methanolic and chloroform) from different plant parts against Gram +ve and Gram-ve bacterial colonies following well diffusion method (Hugo and Russell, 1986). Nutrient Agar media was used (Hi Media, India) for culture of bacteria. The different isolates were dissolved in 100% Dimethylsulphoxide (DMSO) at dose level of 5 mg/mL. Media was cooled and melted at 49 - 51ºC and inoculums (1.5×108 CFU/mL, 0.5 McFarland) were mixed under laminar air flow in media and transferred to autoclaved petri dishes and allowed it for solidification. Wells were punctured in the petridish having solidified media. The samples (100 µl) was poured in the well with micropipettes (at depth of 6 mm). Later on, plates having test sample kept whole night at 37ºC. The potentiality of the extract was calculated against tested clinical strains as clear zone of inhibition covering each well. The diameters were measured in mm along with standard drug which was used as antibiotics in the form of streptomycin. Against each strain controls were used as solvent in the form of respective solvent. The zone of control was deducted from zone of tested sample to determine actual zone were and the final zone was compared with zone of standard. The 3 replicates were processed against each strain to avoid the mistake and the average data were calculated.

Antifungal Assay

Anti fungal potency of both methanolic and chloroform extracts was followed in similar manner with slight modification (Bonjar *et al*., 2005). The microbial fungi colonies were revived in Potato dextrose agar, PDA (Merck, Germany) for their growth and heated next to room temperature for whole night at 25°C for 1-2 days. Inoculums of strains were mixed in autoclaved broth and final concentration was made around 10^6 cells/ml. Petri dish were

dehydrated at 37^0C about 15 min. here also wells were made in autoclaved petriplates. After addition of test sample in the similar manner as mentioned above, plates were heated at 37°C. There after of 1- 2 days same pattern of observations were made (in mm). Complete protocol was repeated thrice and average data were measured.

Antioxidant assay

DPPH assay

This assay done by using spetrophotometer. If DPPH reacts stabilize with scavenging reducing moieties (Brand-Williams *et al.*,1995) [5], it can transfer H^+, it is stabilized. The variation in appereance (deep-violet to light-yellow) noted at 517 nm. Antioxidant potential of extracts was calculated by faintly adapted assay of Brand-Williams *et al*., (1995) [5]. The reaction mixture was prepared prior measurement of absorbance. Plant samples at various concentrations were added in DPPH already dissolved in methanol. Further the reaction mixture was put in absence of light in closed chamber at time interval of about half an hour at $37C^0$. Reduction in absorbance confirms enhanced radical scavenging efficacy. Absorption of blank having concentration of methanol and DPPH mixture was kept ready in same manner. Assay was measured by the below mentioned formula:

%DPPH radical scavenging activity

$$= 100\left(\frac{A_{control} - A_{sample}}{A_{control}}\right)$$

where: A control = absorption of blank (t=0 min);
A sample = absorption of tested sample (t=15 min).

FRAP (Ferric reducing Antioxidant Power)

This protocol involves mechanism of conversion of ferric tripyridyltriazine (Fe (III)-TPTZ) complex into ferrous tripyridyltriazine (Fe (II)-TPTZ) through catalyst in acidic medium. The assay was carried away as per method of Varga *et al*, 1998 [27].

Chemicals Required

- Acetate buffer, 300mM/L pH 3.7 (3.25 g sodium acetate x H_2O and 18 mL conc. CH_3COOH per 1 mL of buffer).
- 10mM TPTZ in 40 mM 1 HCl.
- 20mM $FeCl_3$. 6 H_2O in H2O

FRAP Reagent: 25 mL acetate buffer (1), 2.5mL TPTZ reaction mixture and 2.5mL $FeCl_3$. 6 H_2O. The test samples must be always fresh during experiment. Different doses of Fe (II) concentration was plot as regression curve (dilution having 100-1000µ mol/L).

- **Control: FRAP mixture**

Sample: FRAP mixture +1.5mL, plant extract

Protocol

Tissue (1g) macerated in tiny parts and macerated via chilled mortar and pestle and 9.5 mL pre-chilled 0.1M phosphate buffer mixed. (pH 7.7, having 0.1mM EDTA). The solution was filtered via filter paper and spinned at 16,000 g having time duration of 15 min. The clear extract was taken for calculations. The solution was raised to 5mL and absorbance noted at 593nm.

Estimation: The comparative efficacies of samples were tested by analyzing their standard plot of ferrous sulphate.

MDA

Chemicals required

- Ethanol
- TCA (20% w/v in H2O)
- Butylated hidroxytoluene (0.01% w/v in C2H5OH)
- TBA (0.5% w/v in 20% TCA)

Methodology

This assay is calculated as MDA (malondialdehyde) as by yield of lipid peroxidation as per methodology of Hodges *et al* (1999) [10]. 1g fresh test tissue was macerated in 25 mL of C2H5OH in cool pestle and mortar and spinned at 10000rpm at time interval of 25 minutes at 6ºC. The transparent supernatant considered as enzyme. In first test tube 1mL this clear sample was mixed in 0.8 mL TCA and 0.2 mL of butylated hidroxytoluene was added. In another test tube same sample was added in 0.8 mL TBA having 0.2 mL of butylated hidroxytoluene was again added. The solution heated at 95ºC for 30 min. Reaction saturated via reducing temperature in pre-chilled conditions for 15 min. Reaction mixtures spinned at 10,000 rpm at time interval of 10 min and clear solution were kept to calculate optical density at 532 nm and 400nm. The amount for non-specific optical density at 600 nm was deducted.

Catalase (CAT)

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Chemical required

- Phosphate buffer (50 and 0.1M, pH- 7.2 and 6.8 respectively)
- H_2O_2 (24 mM)
- Na_2EDTA (0.1mM)
- PVP (1%)

Protocol

Here tested tissue (200mg) macerated with 5mL of phosphate buffer along with Na_2EDTA, cooled and spinned at 10000g at duration of 25 min having 6°C. The transparent elutes considered as enzyme. The method has been followed as per described by Aebi (1984) [1] with some variations. 2.0mL of phosphate buffer, 0.8mL of H_2O_2 was mixed and at last 0.2 mL enzyme added followed by instantaneously optical density calculated at 240nm.

Peroxidase Assay (POXA)

The protocol involves chemical conversion of pyrogallol to purpurogallin at time when reaction completed by peroxidase at 420 nm having temperature about 22°C.

$$\text{Pyrogallol} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Purpurogallin}$$

Chemical Required

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (127µmol, pH- 6.9)
- Pyrogallol (55µmol)
- H_2O_2 (32%)

Protocol

Tested tissues (200mg) was macerated in 10mL of phosphate buffer and spinned in cool conditions at 10000 g having time interval of 25 minutes. Again, here clear elutes considered as enzyme. The protocol has been followed as per described by Chance & Maehly (1955) [6] having slight variations. 2.4mL of phosphate buffer, 0.3mL of pyrogallol and 0.2mL of H_2O_2 was mixed. Quantity of purpurogallin observed had been calculated by optical density at 420nm right away later on by mixing 0.1mL enzyme.

Results and Discussion

Antimicrobial activity

While assessing the antibacterial and antifungal efficacy, both methanolic and chloroform extracts leaves and bark of *Derris elliptica* and *Ailanthus excelsa* showed potent antimicrobial activity against clinically microbes. In *D. elliptica* against *E. coli* we found that both chloroform and methanolic extracts of bark showed better activity (IZ-14mm) as compared to leaves. Against *S. aureus* the observations were in contrast to earlier as leaves had better efficacy (IZ -14mm) as compared to bark. Against *P. aeruginosa* highest potency found in methanolic fraction of leaves (IZ -16mm) while against *B. subtilis* partial efficacy was found in some cases.

In fungal efficacy when extracts were tested against *C. albicans* it was observed that maximum efficacy was in methanolic extracts of leaves (IZ-12mm) while in chloroform extracts activity was at par both in leaves and bark. Against *P. chrysogenum* maximum efficacy found in chloroform fraction of leaves (IZ-14mm) while against *A. niger* resistivity was found in both parts. Against *F. moniliforme* maximum efficacy found in chloroform fraction of bark (IZ-16 mm) (Table 1 and 2).

Table 1: Bacterial efficacy of various extracts of *Derris elliptica*

Test organisms		Methanol extract (1mg/ml)		Chloroform extracts (1mg/ml)	
		Bark	Leaves	Bark	Leaves
E. coli	IZ*	14±0.04	10±0.01	14±0.04	12±0.04
	AI*	0.35	0.25	0.35	0.3
S. aureus	IZ*	N.A.	14±0.04	10±0.02	14±0.04
	AI*	-	0.35	0.25	0.35
P. aeruginosa	IZ*	9±0.07	16±0.06	12±0.05	10±0.01
	AI*	0.22	0.4	0.3	0.25±
Bacillus subtilis	IZ*	N.A.	12±0.05	N.A.	10±0.01
	AI*	-	0.3	-	0.25

*IZ = Inhibition zone (in mm) including the diameter of well (6 mm) N.A.- No activity Activity Index (AI) = Inhibition area of the test sample/ inhibition area of the standard Standards: Gentamycin = 1mg/ml (zone -40 mm)

Table 2: Fungicidal efficacy of various extracts of *Derris elliptica*

Test organisms		Methanol extract		Chloroform extracts	
		Bark	Leaves	Bark	Leaves
Candida albicans	IZ*	10±0.01	12±0.02	10±0.01	10±0.01
	AI*	0.33	0.4	0.33	0.33
Penicillium chrysogenum	IZ*	12±0.02	8±0.008	10±0.01	14±0.02
	AI*	0.4	0.26	0.33	0.4
Aspergillus niger	IZ*	14±0.04	14±0.04	N.A.	12±0.02
	AI*	0.46	0.46	-	0.4
Fusarium moniliforme	IZ*	14±0.04	12±0.02	16±0.02	N.A.
	AI*	0.46	0.4	0.53	-

*IZ = Inhibition zone (in mm) including the diameter of well (6 mm) Activity Index (AI) = Inhibition area of the test sample/ inhibition area of the standard Standards: Ketokenazole = 1mg/ml (zone -30 mm)

In *A. excelsa* when extracts were tested against different bacterial strains were studied activity was lower as compared to *D. elliptica*

It was observed that against *E. coli* methanolic extracts of leaves and bark have at par activity (IZ-12 mm) while chloroform extract of bark did not show any activity. Against *S. aureus* maximum activity (IZ-16 mm) was observed in chloroform extracts of leaves while no activity was observed in methanolic extracts.

Against *P. aeruginosa* no activity was observed in any sample so it was found to be resistant while in *B. subtilis* maximum efficacy (IZ-18mm) was observed in methanolic extracts of bark.

In case of fungal efficacy, it was observed that against *C. albicans* both methanolic and chloroform extract of leaves had potent activity while in bark no efficacy was observed. Maximum activity was observed in chloroform extract of leaves (IZ-10mm). Against *P. chrysogenum* except chloroform extracts of leaves no activity was observed in any sample thus it was found to be resistant. Further against *A. niger* moderate efficacy was observed in both chloroform and methanolic extracts of leaves (IZ-8 and 6mm). Against *F. moniliforme* at par efficacy was observed in chloroform extracts of leaves and bark (IZ-12mm each).

(Table3 and 4)

Table 3: Bacterial efficacy of various extracts of *Ailanthus excelsa Roxb*

Test organisms		Methanol extract		Chloroform extracts	
		Bark	Leaves	Bark	Leaves
E. coli	IZ*	12±0.02	8±0.008	N.A.	12±0.02
	AI*	0.3	0.2	-	0.3
S. aureus	IZ*	N.A.	N.A.	10±0.01	16±0.01
	AI*	-	-	0.25	0.25
P. aeruginosa	IZ*	N.A.	N.A.	N.A.	N.A.
	AI*	-	-	-	-
Bacillus subtilis	IZ*	18±0.01	N.A.	N.A.	10±0.01
	AI*	0.25	--	-	0.25

*IZ = Inhibition zone (in mm) including the diameter of well (6 mm) Activity Index (AI) = Inhibition area of the test sample/ inhibition area of the standard Standards: Gentamycin = 1mg/ml (zone -40 mm)

Antioxidant activity

DPPH Activity

In the present investigation by DPPH Assay maximum free radical scavenging activity was observed in leaves of *D. elliptica* (78.87%) while minimum in bark (67.29%) while in *A. excelsa* also similar observations were found (Table 4 and 5).

Table 4: Fungicidal efficacy of various extract of *Ailanthus excelsa Roxb*

Test organisms		Methanol extract		Chloroform extracts	
		Bark	Leaves	Bark	Leaves
Candida albicans	IZ*	N. A	8±0.008	N. A	10±0.01
	AI*	-	0.26	-	0.33
Penicillium chrysogenum	IZ*	N. A	N. A	N. A	8±0.008
	AI*	-	-	-	0.26
Aspergillus niger	IZ*	N. A	6±0.006	N. A	8±0.008
	AI*	-	0.20	-	0.26
Fusarium moniliforme	IZ*	10±0.01	N. A	12±0.02	12±0.02
	AI*	0.33	-	0.4	0.4

*IZ = Inhibition zone (in mm) including the diameter of well (6 mm) Activity Index (AI) = Inhibition area of the test sample/ inhibition area of the standard Standards: Ketokenazole = 1mg/ml (zone -30 mm)

Table 5: DPPH Assay of different plant parts of *Derris elliptica*

S. No	Plant Parts	% DPPH radical scavenging activity
1	Leaves	78.87±2.23
2	Bark	67.29±1.89
	Ascorbic acid	100

Catalase efficacy

In this method we found that in *D. elliptica* potency decreased with increase in time interval till 1 min. Overall leaves have maximum activity (8.81 µM H2O2 reduced/gmfw/sec) as compared to bark (Table 6)

Table 6: DPPH Assay of different plant parts of *Ailanthus excelsa Roxb*

S. No	Plant Parts	% DPPH radical scavenging activity
1	Leaves	62.43±1.76
2	Bark	57.21±1.33
	Ascorbic acid	100

In *A. excelsa* similar observation were recorded as leaves had better activity (7.79 µM H2O2 reduced/gmfw/sec). Over all *D. elliptica* was found to be having better activity as compared to *A. excelsa* (Table7).

Table 7: Catalase Assay of different plant parts of *Derris elliptica* Bark

Time (sec.)	Concentration (µM H_2O2 reduced/gmfw/sec.)
0	-
15	5.87±0.2
30	2.98±0.19
45	2.02±0.17
60	1.54±0.13

Leaf:

Time (sec.)	Concentration (µM H_2O2 reduced/gmfw/sec.)
0	-
15	8.81±0.3
30	4.49±0.2
45	2.99±0.19
60	2.28±0.17

Peroxidase Assay

Here activity was measured in terms of degradation of H_2O_2. In this assay it was observed that in *D. elliptica* activity decreased with increase in time interval till 1 min. Overall leaves have maximum activity (1.24 µM/L/gm dw/sec) as compared to bark (Table 8).

Table 8: Catalase Assay of different plant parts of *Ailanthus excelsa Roxb* Bark

Time (sec.)	Concentration (µM H_2O2 reduced/gmfw/sec.)
0	-
15	4.66±0.21
30	3.02±0.18
45	1.96±0.09
60	1.32±0.08

Leaf:

Time (sec.)	Concentration (µM H_2O2 reduced/gmfw/sec.)
0	-
15	7.79±0.22
30	4.12±0.18
45	2.89±0.09
60	2.07±0.07

In *A. excelsa* similar observation were recorded as leaves had better activity (0.96 µM/L/gm dw/sec). Over all *D. elliptica* was found to be having better activity as compared to *A. excelsa* (Table 9)

Table 9: Peroxidase Assay of different plant parts of *Derris elliptica* Bark

Time (sec.)	Concentration (µM/L/gm dw/sec.)
0	-
15	0.17±0.07
30	0.09±0.008
45	0.07±0.007
60	0.05±0.004

Leaf:

Time (sec.)	Concentration (µM/L/gm dw/sec.)
0	-
15	1.24±0.009
30	0.62±0.006
45	0.42±0.004
60	0.31±0.003

FRAP Activity

In this assay it was observed that in *D. elliptica* activity increased in both plant parts with increase in concentration. Overall leaves have maximum activity (1635 µM) as compared to bark (Table 10) In *A. excelsa* similar observation were recorded as leaves had better activity (1246µM). (Table 11)

Table 10: Peroxidase Assay of different plant parts of *Ailanthus excelsa Roxb* Bark:

Time (sec.)	Concentration (µM/L/gm dw/sec.)
0	-
15	0.15±0.04
30	0.06±0.006
45	0.04±0.004
60	0.03±0.003

Leaf:

Time (sec.)	Concentration (µM/L/gm dw/sec.)
0	-
15	0.96±0.009
30	0.56±0.005
45	0.40±0.004
60	0.37±0.003

Table 11: Ferric Reduction Antioxidant power (FRAP) Activity of *Derris elliptica* Bark:

Sample (µg/ml)	Concentration (µM)
20	118.09±2.83
40	183.54±3.01
60	222.63±3.67
80	259.90±3.88
100	266.27±3.98

Leaf:

Sample (µg/ml)	Concentration (µM)
20	759±5.67
40	1050.8±8.56
60	1506.27±9.3
80	1584.45±10.1
100	1635.36±10.5

Table 12: Ferric Reduction Antioxidant power (FRAP) Activity of *Ailanthus excelsa Roxb* Bark:

Sample (µg/ml)	Concentration (µM)
20	93.04±1.82
40	171.87±2.56
60	216.67±2.87
80	273.32±2.94
100	281.64±2.98

Leaf:

Sample (µg/ml)	Concentration (µM)
20	663.87±4.67
40	988.23±7.87
60	1498.31±8.37
80	1386.24±7.56
100	1246.77±6.56

Over all *D. elliptica* was found to be having better activity as compared to *A. excelsa* in terms of antioxidant efficacy when measured by above all assays. Bioactive compounds wide array of organic molecules as carbohydrates are initial group of metabolites which is formed during organic molecules in the plants due to biochemical reaction of photosynthesis, also provides energy in form of ATP. Besides this, phytochemicals are engaged as crucial scaffold or they also cause variation in the physico-chemical features in other compounds by reacting with them.

Production of free radicals leads in several metabolic disorders. Therefore, estimation of free radical scavenging activity in plants ultimately results in innovation of natural antioxidants with prominent efficacy having nutritive importance (Ao *et al*., 2008) [2]. Role of phenolic compounds is to act as free radical scavengers and their application in replacement of synthetic moieties in food supplements has been documented (Marhoume *et al*., 2021) [16]. Therefore, based on this data we could assist in evolving novel medicines in biomedical application in human-beings. However, only scanty reports are available has been done in some plant species and their exact mode of action is still to be elucidated

DPPH Activity

In the present investigation by DPPH Assay maximum free radical scavenging activity was observed in leaves of *D. elliptica* (78.87%) while minimum in bark (67.29%) while in *A. excelsa* also similar observations were found.

Catalase Activity

In this assay it was observed that in *D. elliptica* activity decreased with increase in time interval till 1 min. Overall leaves have maximum activity (8.81 µM H2O2 reduced/gmfw/sec) as compared to bark.

In *A. excelsa* similar observation were recorded as leaves had better activity (7.79 µM H2O2 reduced/gmfw/sec). Over all *D. elliptica* was found to be having better activity as compared to *A. excelsa.*

Here activity was measured in terms of degradation of H_2O_2.

Peroxidase Activity

In this assay it was observed that in *D. elliptica* activity decreased with increase in time interval till 1 min. Overall leaves have maximum activity (1.24 µM/L/gm dw/sec) as compared to bark.

In *A. excelsa* similar observation were recorded as leaves had better activity (0.96 µM/L/gm dw/sec). Over all *D. elliptica* was found to be having better activity as compared to *A. excelsa*

FRAP Assay

In this assay it was observed that in *D. elliptica* activity increased in both plant parts with increase in concentration. Overall leaves have maximum activity (1635 µM) as compared to bark

Our physiological mechanism possesses many defense modes of action against reactive oxygen species, which involve defense mechanism, regeneration of cells of action, physical defenses and scavenging activity against free radicals. ROS has attracted keen interest from scientific communities as it has environmental influence as drought, cold, high, temperature, herbicides and heavy metals, as they disrupt living cells by increasing free radicals by damage of morphology and role (Oke & Hamburger, 2002) [18].

Telekone & Khan 2014 [26], reported Antiinflammatory and free radical scavenging efficacy of various plants parts from *Derris brevipes.* They reported that IC_{50} for these efficacies was least in methanol sample and compound 2 in contrast to chloroform extract and compound1.

Paul *et al* (2019) [20] reported pharmacological activity screening of *Derris robusta*.

They reported the O.D. of tested samples around 0.349 and TPC content around 43.17 GAE/g which was less than our present research proves that the plant has free radical scavenging assay. The amount of total flavonoid in experimental plants was analysed through spectrophotometer by aluminium chloride colorimetric method. The flavonoid amount calculated as mg quercetin calculated in terms of g of extract was 192.58 QE/g indicates present of flavonoid content was more than in our present research. In DPPH the IC_{50} of methanolic samples was 91.45 µg/ml which was less than our present research.

Antimicrobial assay

Disease caused due to microbes is leading outcome in the form of morbidity and death globally. The amount of MDR colonies and mutation in strains which decreased vulnerability to antibiotics are incessantly enhanced. This elevation is correlated to haphazard application of expansive gamut synthetic drugs, immunosuppressive molecules, intravenous organ transplantation and currently outer layer of human immunodeficiency virus (HIV) disorders. Mutation of multidrug defiant microbes is hazardous globally so it is tedious task to combat their threatening infection. Further, the present amount of most of the synthetic antibiotics is very expansive which is difficult to be affordable by middle class family or lower class in asian nations like India (Sarala *et al*., 2010) [22]. So, there is an urge towards the intend and innovation of prominent herbal, effective medicines for behavior and proved belief urge for novel antimicrobial agents based on herbal drugs

They are rich deposits of natural products which could act as effective of antimicrobial agents. The initial phase studies are to go through *in vitro* antimicrobial assay. Lot of research have been done on efficacy of natural products against virus, microbes, protozoans, parasites and anti-inflammatory isolated from herbal drugs (Govindarajan *et al*., 2006) [8]. Few reports have assisted for screening of the phytochemicals associated with many efficacies including designing medicines for the pharmacological application in human beings. Lot of reports are available which proves antimicrobial efficacy of medicinal plants (Ibekwe *et al*., 2000) [11].

While assessing the antibacterial and antifungal efficacy, both methanolic and chloroform extracts leaves and bark of *Derris elliptica* and *Ailanthus excelsa* showed potent antimicrobial activity against clinically microbes. In *D. elliptica* against *E. coli* we found that both chloroform and methanolic fractions of bark showed better activity (IZ-14mm) as compared to leaves. Against *S. aureus* the observations were in contrast to earlier as leaves had better efficacy (IZ -14mm) as compared to bark. Against *P. aeruginosa* highest efficacy found in methanolic fraction of leaves (IZ -16mm) while against *B. subtilis* no activity was observed.

In efficacy against fungal pathogens when extracts were tested against *C. albicans* it was observed that maximum efficacy was in methanolic extracts of leaves (IZ-12mm) while in chloroform extracts activity was at par both in leaves and bark. Against *P. chrysogenum* highest potential was observed in chloroform fraction of leaves (IZ-14mm) while against *A. niger* no activity was observed in chloroform extracts of both parts. Against *F. moniliforme* maximum efficacy found in chloroform fraction of bark (IZ-16 mm). In *A. excelsa* when extracts were tested against different bacterial strains were studied activity was lower as compared to *D. elliptica*

It was observed that against *E. coli* methanolic extracts of leaves and bark have at par activity (IZ-12 mm) while chloroform extract of bark did not show any activity. Against *S. aureus* maximum activity (IZ-16 mm) was observed in chloroform extracts of leaves while no activity was observed in methanolic extracts.

Against *P. aeruginosa* no activity was observed in any sample which proves its sensitivity while in case of *B. subtilis* maximum efficacy (IZ-18mm) was observed in methanolic extracts of bark.

In case of fungal efficacy, it was observed that against *C. albicans* both methanolic and chloroform extract of leaves had potent activity while in bark no efficacy was observed. Maximum activity was observed in chloroform extract of leaves (IZ-10mm). Against *P. chrysogenum* except chloroform extracts of leaves no activity was observed in any sample thus it was found to be resistant. Further against *A. niger* moderate efficacy was observed in both chloroform and methanolic extracts of leaves (IZ-8 and 6mm). Against *F. moniliforme* at par efficacy was observed in chloroform extracts of leaves and bark (IZ-12mm each).

Khan *et al* (2006) [13] proved efficacy against microbes from *Derris elliptica*, *Derris indica* and *Derris trifoliata* parts. They reported prominent efficacy in methanolic pet ether and butanol elutes of leaves and root heart-wood including bark, of *D. indica* and pet ether and ethyl acetate elutes of *D. trifoliata*. No fungal activity was reported in these extracts.

They reported that these compounds have potent antifungal efficacy against *Candida albicans* and partial activity against bacterial strains. Some rotenoids showed partial inhibition on augmentation of *Enterococcus faecalis*, *Staphylococcus aureus* and *C. albicans*.

Shrimali *et al* (2001) [25] proved antibacterial potential of *Ailanthus excelsa*. They reported that the ethyl acetate extracts have potential efficacy against bacterial strains. The MIC of ethyal acetate extracts was around 6 mg/disc.

Sharma *et al* (2023) [24] reported physico chemical evaluation and antimicrobial efficacy of bark of *Ailanthus excelsa*. They proved that various sequential extracts like Methanol, chloroform and ethyl acetate showed potent antibacterial and antifungal efficacy and n hexane fraction did not show any efficacy but overall activity was less than our present research.

Pal *et al* (2023) [19] reported biochemical estimation of seed oil and oil cake of *Ailanthus excelsa*. They reported that at dose level of 100 µL, the no antibacterial efficacy was observed against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Similarly, no antifungal efficacy was observed against *Candida albicans* and *Aspergillus flavus.*

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