In Vitro Evaluation of Antimicrobial Activity of Fractions of Delonix Regia Leaf Extracts

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Abstract

*The objective of the present investigation was to assess the antimicrobial potential of Delonix regia leaf extract fractions. The crude extracts (chloroform and methanolic) of the leaves of the plant were subjected to fractionation using n-hexane, ethylacetate and methanol. The fractions obtained (A-F) were tested for phytochemicals and the confirmation of the same were achieved by thin layer chromatographic analysis. The in vitro antimicrobial activity was done using cup and plate method using Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus niger. The maximum antibacterial activity was exhibited by fractions **E** and **F** at concentration of 400 µg while fractions **A** and **D** were significantly effective against the fungal strain. The findings of the study provide a scientific basis to further investigate the potential of the leaves of D. regia.*

Key-words*: Delonix regia, Anti-microbial activity, Leaf Extract*

Introduction

The use of plants for treatment of ailments of mankind has been dated back several centuries. The scientific exploration of the folklore remedies has resulted in several potent drug molecules. Despite their vast potential only a small percentage of these folklore medicines have been subjected to phytochemical investigation and fractionated for pharmacological screening. The plants used in traditional systems of medicine are easily found in the rural areas and offer an economically viable alternative to the modern medicine.

Delonix regia, found as an ornamental tree in several regions of the world, is a species of the flowering plant of family Fabaceae, subfamily Caesalpinioideae. It is widely called as flamboyant, flame of the forest, Gulmohar or Royal Poinciana. The tree has fern-like leaves and red colored peacock flowers. The flowers, leaves and barks have been reported to possess the majority of active constituents of the plant. The flowers have insecticidal [1], wound healing [2], anthelmintic activities [3] and have also inhibited the malaria parasite [4]. The leaves of *D. regia* are known to possess anti-inflammatory [5], antiulcer [6], antifungal [7], and cytotoxic actions [8]. Therefore an attempt has been made to evaluate the antimicrobial activity of various solvent fractions of the chloroform and methanolic extracts of *D. regia*.

Material and Methods

Plant Material

The leaves of *Delonix regia* were collected from the trees growing in the region of Bhopal in the month of October 2019. The plant was taxonomically identified at the botany department of MFP-PARC, Bhopal.

The leaves were dried under shade, coarsely powdered and passed through sieve number 40 and stored in closed container prior to extraction.

Extraction and fractionation

500 g of the leaf powder was evenly packed in the extractor of the soxhlet apparatus and extracted successively with solvents of increasing polarity including petroleum ether, chloroform and methanol by hot continuous extraction process for about 27 h. The aqueous extraction was carried out using cold maceration method. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation under reduced pressure to obtain the residue [9].

The crude chloroform and methanolic extracts were separately fractionated with several potions of n-hexane, ethylacetate and methanol successively using glass column packed with silica gel (100-200 mesh) [10]. The fractions were collected and monitored using TLC performed on Silica gel G precoated TLC plates using n-hexane- ethyl acetate (80:20) (S_1), chloroform-methanol (80:20) (S_2) and ethyl acetate-chloroform-water (100:16.5:13.5) (S_3) as the solvent systems. These fractions were evaporated to obtain the residues **A**, **B** and **C** from n-hexane, ethylacetate and methanol fractions respectively of the chloroform extract and **D**, **E** and **F** from the same solvent fractions of the methanolic extract. All the fractions were subjected to phytochemical tests to assess the class of chemical constituents present in them [11].

Antimicrobial Screening

Microorganisms tested

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Escherichia coli* (MTCC 42), *Bacillus subtilis* (MTCC 736), *Staphylococcus aureus* (MTCC 10787), *Pseudomonas aeruginosa* (MTCC 8077) and *Aspergillus niger* (MTCC 11098) were used for the present investigation.

The lyophilized cultures were revived by adding 0.3mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria. Revival of the fungal culture was done using 0.3mL of water.

Screening Procedure

About 3mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. Potato dextrose agar plate was used for inoculating the fungus under examination. The antimicrobial action was screened using disc diffusion method [12]. Fractions **A, B, C** were dissolved in chloroform while **D, E & F** were dissolved in methanol to obtain 1mg/mL, 1.5 mg/mL and 2mg/mL solutions of each. Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the fractions were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters and the average diameter of the zone of inhibitions was calculated. In case of the fungal plate, the zone of inhibition was measured after 72h of incubation at 30°C. Chloramphenicol and Ketoconazole were used as the standards for antibacterial and antifungal activities respectively. The activity index was calculated by subtracting the diameter of the well from the diameter of the zone of inhibition and dividing the result by the diameter of the well.

Results and Discussion

Extraction Yield

The extraction yield of the crude extracts obtained using various solvent' is calculated as the percentage of dry weight of the powdered leaves and is presented in Figure 1. It was observed that methanol and chloroform exhibited comparatively higher extraction efficiency than petroleum ether and water. The ability of the solvents to recover the extractable components of the leaves followed the order: methanol (41.6%)>chloroform (21.3%)>water (12.4%)>petroleum ether (3.2%). In a previous study by Shabir et al [13], they reported that 80% methanol was able to extract the highest amounts of constituents of *D. regia* leaves. In another study initiated by Wang et al [14], the yield of ethanolic extract of the leaves of the plant was found to be 13.8%.

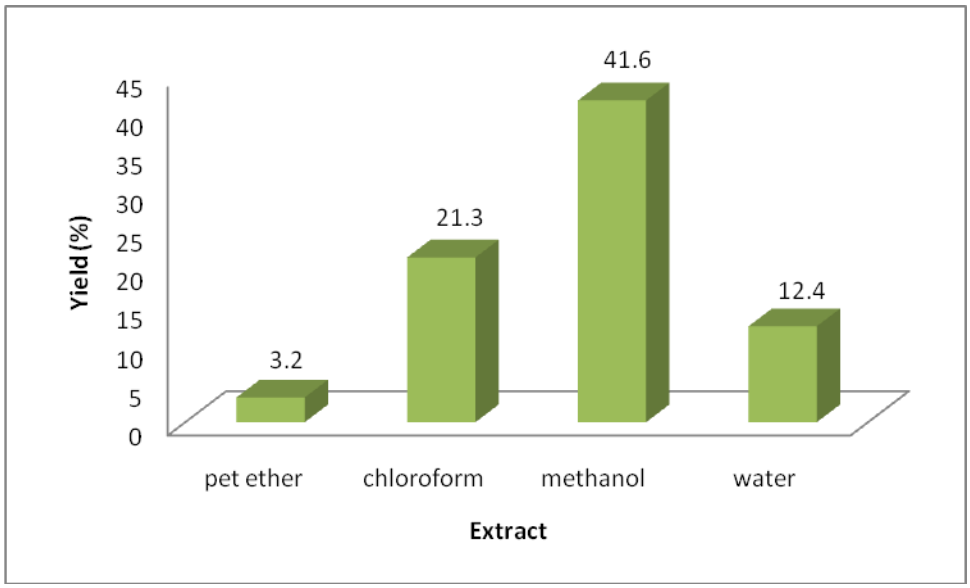

Figure 1: Extraction yield (% of dry weight) from *Delonix regia* leaves

Fractionation of extracts

Amongst all the solvents, methanol and chloroform were able to recover the highest amounts of extractable material from the leaf powder and hence these two solvents were subsequently subjected to fractionation using n-hexane, ethylacetate and methanol. The n-hexane fractions were considered to be containing the most non-polar constituents including sterols, while the ethylacetate and methanolic fractions were hypothesized to be flavonoid rich. The TLC of all the fractions is depicted in Figure 2 and the result of phytochemical screening of the fractions is presented in Table 1.

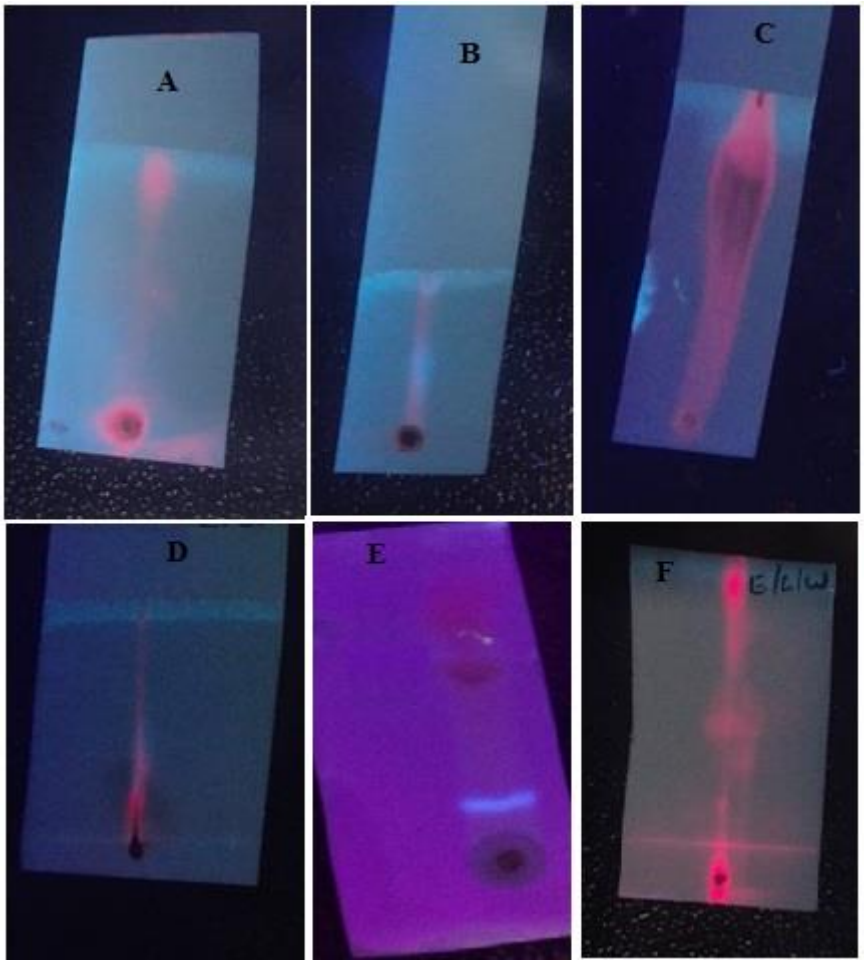

Figure 2: TLC of fractions A (S_1), B (S_1), C (S_2), D (S_1), E (S_2), F (S_3)

The qualitative tests of the fractions exhibited positive results for the presence of sterols (**A, B, D**); alkaloids (**C, F**), flavonoids, saponins and tannins (**B, C, E, F**). The TLC profile of the fractions also revealed multiple components in the fractions. Mariajancyrani et al [15] have also previously reported the leaves of *D. regia* 95% ethanolic extract to contain tannins, terpenoids, flavonoids, steroids and fatty acids.

Table 1: Phytochemical screening of the fractions

Chemical Tests	Observation to be made	Choloroform extract			Methanolic extract		
		Fraction A	Fraction B	Fraction C	Fraction D	Fraction E	Fraction F
Alkaloids							
Mayer's reagent	cream colour precipitate	-	-	-	-	-	-
Dragendorff's reagent	reddish brown precipitate	-	-	+	-	-	+

Glycosides							
Froth test	Frothing	-	+	+	-	+	+
Bontrager's Test	Rose pink or red color in the ammonical layer	-	-	-	-	-	-
Phenols/Tannins							
Ferric chloride	Blue green color	-	+	+	-	+	+
Vanillin HCl test	Purplish red color	-	+	+	-	+	+
Flavonoids							
Shinoda test	red color	-	+	+	-	+	+
Zinc HCl reductino test	red color	-	+	+	-	+	+
Sterols/triterpenoids							
Liberman-Burchard Test	Brown ring at junction Upper layer turns green	+	-	-	+	-	-
Salkowski Test	Yellow color in lower layer	+	-	-	+	-	-

Antimicrobial Activity

The antimicrobial activity of all the fractions was assessed at three concentration levels using cup and plate method. The activity varied significantly in the fractions. Fraction **E** and **F** were found to exhibit the most effective antimicrobial action against the bacterial (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa)* and while fractions **A** and **D** were found to be most effective against the fungal (*Aspergillus niger)* strains. The zone of inhibition of the positive control was significantly higher than most of the tested fractions. The activity index was calculated to ascertain the susceptibility of the microbial strains against the fractions. The results obtained from the antimicrobial evaluation are presented in Table 2.

The fractions were found to inhibit microbial growth in a concentration dependent manner. The maximum activities were exhibited by fractions **E** and **F** at concentration of 400 µg. The gram-positive bacteria (*B. subtilis and S. aureus*) were found to be more susceptible to the extracts as compared to the gram-negative bacteria (*E.coli and P. aeuroginosa*). The zone of inhibition of fraction **E** and **F** at 400 µg concentration was 23.67±0.8819 (*E.coli*) with AI 1.367, 24.67±0.8819 (*B. subtilis)* with AI 1.467, 19.67±0.6667 (*P. aeuroginosa*) with AI 0.967 and 25.00±0.5774 (*S. aureus*) with AI of 1.5. The fractions **E** and **F** though were found to be ineffective against the fungal strain under examination. The zone of inhibition of the standard drug chloramphenicol at 30µg concentration was found to be 29.67±1.202, AI

1.967; 30.00±2.082, AI 2; 26.67±1.202, AI 1.667 and 29.00±2.000, AI 1.9 respectively for the above strains.

On the other hand, it was found that the fractions **A** and **D** that contained sterols were significantly effective against the fungal strain (*A. niger*) with zone of inhibition of 17.67±0.3333, AI 0.767 and 20.33±1.453, AI 1.033 at 400 µg concentration while the zone of inhibition exhibited by the standard drug Ketoconazole (30 µg) was found to be 23.33±0.8819, AI 1.333. The other fractions were not able to inhibit the growth of the fungal strain in the culture.

It could be inferred from the antimicrobial study that the fractions of the methanolic extract of *D. regia* leaves that were rich in flavonoids were more effective in inhibiting the growth of bacteria while the non-polar fractions of both chloroform and methanolic extract, containing sterols, was able to contain the growth of fungus.

Table 2: Antimicrobial activity of fractions of *Delonix regia*

Fraction	Concentration (µg)	*E. coli*		*B. subtilis*		*P. aeuroginosa*		*S. aureus*		*A. niger*	
		ZoI (mm)*	**AI**	**ZoI (mm)***	**AI**	**ZoI (mm)***	**AI**	**ZoI (mm)***	**AI**	**ZoI (mm)***	**AI**
A	200	12.33±0.3333	0.233	15.33±0.8819	0.533	11.67±0.8819	0.167	15.00±1.000	0.5	13.67±1.202	0.367
	300	16.33±0.3333	0.633	17.67±1.202	0.767	13.67±0.8819	0.367	17.67±1.667	0.767	16.00±1.528	0.6
	400	17.67±0.3333	0.767	19.67±0.6667	0.967	15.67±0.3333	0.567	20.00±0.5774	1	17.67±0.3333	0.767
B	200	12.00±0.5774	0.2	16.00±1.000	0.6	12.67±0.8819	0.267	16.33±0.6667	0.633	10.33±0.3333	0.033
	300	15.67±0.3333	0.567	16.67±1.453	0.667	14.67±0.8819	0.467	16.67±1.202	0.667	11.00±0.5774	0.1
	400	18.00±0.5774	0.8	20.33±0.6667	1.033	16.67±0.3333	0.667	20.33±0.3333	1.033	11.33±0.8819	0.133
C	200	12.33±0.3333	0.233	17.00±0.5774	0.7	13.33±0.6667	0.333	17.67±0.8819	0.767	11.67±0.3333	0.167
	300	16.33±0.3333	0.633	18.67±0.8819	0.867	16.33±0.8819	0.633	19.00±1.000	0.9	12.00±0.5774	0.2
	400	18.67±0.3333	0.867	21.33±1.202	1.133	16.33±0.6667	0.633	21.67±0.8819	1.167	12.33±0.3333	0.233
D	200	12.33±0.6667	0.233	15.33±0.8819	0.533	12.0±1.0000	0.2	15.67±1.453	0.567	15.00±1.0000	0.5
	300	16.67±0.8819	0.667	18.33±0.8819	0.833	15.67±0.8819	0.567	19.33±0.8819	0.933	18.67±1.453	0.867
	400	17.33±1.202	0.733	19.67±0.8819	0.967	17.33±0.8819	0.733	20.67±0.6667	1.067	20.33±1.453	1.033
E	200	16.67±0.3333	0.667	18.00±0.5774	0.8	13.67±0.6667	0.367	19.33±0.6667	0.933	11.00±0.5774	0.1

	300	19.33±0.3333	0.933	21.33±0.3333	1.133	16.67±0.3333	0.667	22.33±0.3333	1.233	10.67±0.6667	0.067
	400	23.67±0.8819	1.367	24.67±0.8819	1.467	19.67±0.6667	0.967	25.00±0.5774	1.5	11.67±0.3333	0.167
F	200	16.33±0.6667	0.633	17.67±0.8819	0.767	13.67±1.202	0.367	18.00±1.0000	0.8	11.00±0.5774	0.1
	300	19.33±0.6667	0.933	21.67±0.3333	1.167	18.67±0.8819	0.867	22.33±0.3333	1.233	11.67±0.3333	0.167
	400	22.67±0.3333	1.267	23.67±0.3333	1.367	20.67±0.8819	1.067	24.33±0.3333	1.433	11.67±0.8819	0.167
Chloramphenicol	30	29.67±1.202	1.967	30.00±2.082	2	26.67±1.202	1.667	29.00±2.000	1.9	-	-
Ketoconazole	30	-	-	-	-	-	-	-	-	23.33±0.8819	1.333

Conclusion

On examining the results obtained from the present investigation, it could be inferred that the leaves of *Delonix regia* are a rich source of the secondary metabolites and are therefore of great medicinal help. TLC profiling of the fractions obtained from solvent elution of the chloroform and methanolic extracts was confirming the presence of alkaloids, sterol and flavonoids in the leaves. The antimicrobial action of the fractionated extract was comparable to that of existing antimicrobial drugs especially against the gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Isolating a pure compound from the ethylacetate or methanolic fraction of the methanolic extract of the leaves of *Delonix regia* might provide even better antibacterial action. Further investigation would be helpful in undermining the action of the plant against viruses and parasites.

References

1. Baskaran V, Narayansamy P. Traditional Pest control. Caterpillar Publications, 1995: 190.
2. De Groot H. Reactive oxygen species in tissue injury. Hepatogastroenterology, 1994, 41(2): 328-332.
3. Gupta RK, Chandra S. Chemical investigation of *Delonix regia* flowers. Ind J Pharm, 1971, 33(4): 74-75
4. Carter R, Diggs CL. Plamodia of rodents. In: Parasitic Protozoa. London: Academic Press, 1977: 359-465.
5. Sethuraman MG, Sulochana N. The anti-inflammatory activity of *Delonix elata*. Curr Sci, 1986, 55: 343.
6. Goel RK, Pandey VB, Dwivedi SPD, et al. Antiinflammatory and antiulcer effect of kaempferol, a flavone isolated from *Rhamnus procumbens*. Ind J Exp Biol, 1998, 26: 121-124.
7. Pandey DK, Chandra H, Tripathi NN. Volatile fungitoxic activity of some higher plants with special reference to that of *Callistemon laceolatus* DC. J Phytopathol, 1982, 105(2): 175-182.
8. Azab SS, Abdel-Daim M, Eldahshan OA. Phytochemical, cytotoxic, hepatoprotective and antioxidant properties of *Delonix regia* leaves extract. Med Chem Res, 2013, 22: 4269-4277.
9. Panigrahy SK, Kumar A, Bhatt R. Antioxidant potentials of successive solvent extracts from the unexplored *Hedhychium coronarium* rhizome. J Food Sci Technol, 2017, 54(10): 3297-3306
10. Venkatesh U, Javarasetty C, Murari SK. Purification and fractional analysis of methanolic extract of *Wedelia trilobata* possessing apoptotic and anti-leukemic activity. Afr J Tradit Complement Altern Med, 2017, 14(3): 167-174.
11. Mehta S, Singh RP, Pooja S. Phytochemical Screening and TLC Profiling of Various Extracts of *Reinwardtia indica*. Int J Pharmacog Phytochem Res, 2017, 9(4): 523-527.
12. Manandhar S, Luitel S, Dahal RK. *In Vitro* Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. J Tropical Med, 2019, Article ID 1895340.
13. Shabir G, Anwar F, Sultana B, Khalid ZM, Afzal M, Khan QA, Ashrafuzzaman M. Antioxidant and Antimicrobial Attributes and Phenolics of Different Solvent Extracts from Leaves, Flowers and Bark of Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf.]. Molecules, 2011, 16: 7302-7319.
14. Wang L-S, Lee C-T, Su W-L, Huang S-C, Wang S-C. *Delonix regia* Leaf Extract (DRLE): A Potential Therapeutic Agent for Cardioprotection. PLoS ONE, 2016, 11(12): e0167768. doi:10.1371/journal.pone.0167768

15. Mariajancyrani J, Chandramohan G, Saravanan P, Saivaraj S. *In-Vitro* Antioxidant Potential of *Delonix regia* Leaves. Sch Acad J Pharm, 2013, 2(6): 468-471.