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INVESTIGATION OF PHYTOCHEMICAL, ANTI MICROBIAL ACTIVITIES OF *JUSTICIA GENDARUSSA* AND *JUSTICIA ADHATODA*

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Abstract

The aim of the study was to explore the Phytochemicals (Alkaloids, Flavonoids, Terpenes, Tannins, Saponinsand Phenols) in root, stem and leaves of *Justicia gendarussa* and *Justicia adhatoda* and to correlate it with inhibition of microbes.Alkaloids, flavonoids and phenols were present in both the selected plants while saponins were not detected in the same plants.All fractions of root stem and leaves samples of *Justicia gendarussa* were active against the selected bacterial strains *Enterococcus faecalis, Salmonella typhii, Proteus mirabilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* except *n*-hexane fraction of the plant which was fund inactive. Among fractions obtained from root of *Justicia gendarussa*, the chloroform fraction showed highest zone of inhibition 27 mm against *Staphylococcus aureus.* While the crude extract and fractionsderived from different parts of *Justicia adhatoda* showed moderate to good activities against all tested bacteria giving range of inhibition zones from 14 mm to 28 mm. our research plants also showed good antifungal activities against the four tested fungi.

Key words: Medicinal plants, Phytochemical, Antimicrobial, *Justicia gendarussa* and *Justicia adhatoda*.

Introduction

Due to inadequate medical amenities in the rural areas of the developing countries including Pakistan, a lot oftherapeutic plants are conventionally used for the curing of diseases like diarrhea, skin infections, malaria, diabetes, respiratory problems, fungal and antibacterial infections (Afzal *et al.,* 2013; Hamza *et al.,* 2020). Although thousands of medicinal plants have been evaluated for antimicrobial properties but still the vast majority of which have not been satisfactorily screened. Plants that have own curative properties and exert worthwhile pharmacological effects on the human body are often designated as medicinal plants. Secondary metabolites/ Phytochemicals like alkaloids, sterols, tannins, resins, lactones, flavonoids, saponins, glycosides, volatile oils etc are naturally synthesized and conglomerate in medicinal plants. These are the biologically active substances that have a wide range health benefits for human beings other than those imputed to macronutrients and micronutrients. Plants synthesize these chemical substances to fortify themselves, but recent researches shows that these chemical substances can protect humans against diseases (Mahesh & Satish, 2018*;* Ullah *et al.,* 2017; Phillipson, 1999)**.** The issue regarding microbial resistance towards drugs is of high concern. Therefore, it is necessary to reduce the use of antibioticand to continue studies to develop new novel natural drugs. The eventualtarget is to offer suitable antimicrobial drugs to the patients in form of medicinal plants (Tariq *et al.,* 2016; Ahmad *et al.,* 2019). *Justicia gendarussa* belongs to family Acanthaceae, is a swift growing shrub found in almost all Asian countries like Pakistan, India, Sri Lanka, Indonesia and Malaysia (Yaseen *et al., 2019;* Gislene *et al.,* 2000; Khan *et al.,* 2018). The different parts of *J. gendarussa* are used for curing various diseases like arthritis, rheumatism, earache, hemiplegia, headache etc (Javid *et al.,* 2009). *Justicia adhatoda*is often known as *Adhatoda vasica*. The roots, leaves, flowers and fruit are largely used for treatingchronic bronchitis,cold cough, whooping cough, asthma, expectorant, as sedative and Antispasmodic (Atul & Ghosh, 2014). Considering the vast potential of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic exploration was undertaken to evaluate the medicinal plants *Justicia gendarussa* and *Justicia adhatoda* for Phytochemical and antimicrobial activities.

Materials and Methods

Collection and drying of plant materials: Adequate quantity of *Justicia gendarussa* and *Justicia adhatoda* plants were collected from Goddikhel, District Karak, Khyber Pakhtunkhwa, Pakistan and were identified by Nisar Ahmad Lecturer Department of Botany, Kohat University of Science & Technology, Kohat, Pakistan. Plants were appropriately rinsed with distilled water to purge dust, dirt and other possible parasites and then were shade dried at 25-30^{o}C. The dried parts root, stem, and leaves were pulverized incoherently and then storedin clean, dried plastic bags for extraction.

Extraction procedure: The stored root, stem and leavespowder of weight 2 Kg each of *Justicia gendarussa* and *Justicia adhatoda* was taken and drenched in ethanol for two weeks separately and were extracted at room temperature in the same solvent and then filtered. The filtrates were evaporated under reduced pressure by vacuum rotary evaporator at 35^{0}C to obtain crude extracts i.e. *Justicia gendarussa* (190 gm) and *Justicia adhatoda* (175 gm). These extracts were further suspended in water

and partitioned successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to obtain their solvent soluble fractions. The crude extract of *Justicia gendarussa* on solvent-solvent fractionation yielded *n*-hexane fraction (19 gm), chloroform fraction (37 gm), ethyl acetate fraction (23 gm), *n*-butanolfraction (24 gm)and aqueous fraction (67 gm) while the solvent-solvent fractionation of crude extract of *Justicia adhatoda* provided *n*-hexane fraction (17 gm), chloroform fraction (33 gm), ethyl acetate fraction (21 gm), *n*-butanol fraction (25 gm) and aqueous fraction (61 gm).

Antimicrobial procedure: Crude and other solvent soluble fractions of *Justicia gendarussa* and *Justicia adhatoda* were evaluated for bacterial strains like *Enterococcus faecalis, Salmonella typhi, Proteus mirabilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia* and fungal strains *Aspergillus niger, Aspergillus flavus, Fusarium solani* and *Aspergillus fumigates.*

Antibacterial bioassay: Agar Well Diffusion Method was used to checkantibacterialactivities of our research plants against the testedmicroorganisms by using standard protocol of Asghari with a little amendment (Ahmad *et al.,* 2019). Amoxicillin (5 µg/µL) and Levofloxacin (5 µg/µL) were used as positive control while DMSO was used as a negative control. The zones of inhibitions of our plantextracts/fractions were compared with inhibitionzones of standard drugs.

Antifungal bioassay: Antifungal bioassay was conducted by Agar Tube Dilution Method by using Atta *et al.,* (2001) method with some alteration (Yaseen *et al.,* 2019). Solutions of 2µg/µL concentration of crude extracts and sub fractions were prepared. The standard drug, Clotrimazole dissolved in distilled water (2µg/µL) was used as a positive control while Clotrimazole dissolved in DMSO (6µl/disc) was used as anegative control, respectively.

Phytochemical investigations: Phytochemical study was carried out using standard procedure to identify the constituents as described by Harborne and Sofowora for the exposure of alkaloids, flavonoids tannins, terpenes, saponins and phenols.

Alkaloids: Diluted 70% HCl solution was added vigorously to extracts and fractions. After filtration, saturated solution of picric acid (Hager's reagent) was mixed with the filtrate. The presence of alkaloids was confirmed by yellow color precipitate formation (Gislene *et al.,* 2000).

Flavonoids: Each sample was mixed with 10 ml of ethyl acetate, heated and filtered.Added 1ml diluted ammonia solution to 4 ml filtrate. The presences of flavonoids were confirmed by yellow color precipitate formation (Khan *et al.,* 2018).

Tannins: The samples were separately heated with 20 ml of distilled water for 5 minutes and hot solution was filtered. Then 5 ml of distilled water and 2, 3 drops of 10% ferric chloride were added to 1 ml of filtrate. Formation of bluish-black or brownish-green precipitates indicated the existence of tannins (Javid *et al.,* 2009).

Terpenes: Specific amount of extracts and fractions were added separately to water followed by a few drops of ethyl acetate. Appearing of bright green color indicated the occurrence of terpenes in the samples (Atul & Ghosh, 2014).

Saponins: Extracts and fractions wereunconnectedly heated with 10ml of distilled water for 10minutes. Hot mixture was filtered and allowed to cool down. Then 3 ml of filtrate was added to 10 ml distilled water and shaken vigorously for 2 minutes. Froth formation in the filtrate indicated the presence of saponins (Asghari *et al.,* 2006).

Phenols: Few drops of 0.1% FeCl3 solution was added to extracts and fractions one by one. The presence of phenol was confirmed by black or bluish color formation (Atta *et al.,* 2001).

Results and Discussion

Phytochemical analysis: Phytochemical analysis of crude extracts and sub fractions of *Justicia gendarussa* and *Justicia adhatoda* shows that Alkaloids, flavonoids, tannins and phenol were there in all the three parts, root stem and leaves of *Justicia gendarussa* while terpenes and saponins were not detected in any part of the selected plant *Justicia gendarussa* (Tables 1 & 2). The selected Phytochemicals were not recorded in *n*-hexane fractions. The various parts of *Justicia adhatoda* plants were enrich of Alkaloids, Flavonoids, terpenes and phenol while Tannins and saponins were lacked in the same plant.

Antimicrobial analysis

Antibacterial activity: Different zone of inhibitions were shown by extracts and fractions of roots, stems and leaves of *Justicia gendarussa* and *Justicia adhatoda* with different concentrations 2µg/µl and 4µg/2µl (Tables 3 & 4). The extracts / fractions obtained from our selected plants were screened against different bacterial strains. All fractions of root stem and leaves samples were found active against the selected microbes showing good activities except *n*-hexane fraction of the plants which was found inactive. Chloroform fractionof the plant showed high zone of inhibition 27 mm against *Staphylococcus aureus n*-butanol fraction of stem was only active against *Enterococcus faecalis* while in case of leaves fractions of *Justicia gendarussa,* all fractions were inactive against *Proteus mirabilis* and *Escherichia coli.*

Similarly the different fractions of root, stem and leaves of *Justicia adhatoda* were evaluated for antibacterial activities and zone of inhibition in mm was recorded. Chloroform fraction of *Justicia adhatoda* (root) showed best activity against all the selected microbes. Chloroform, Ethyl acetate and *n*- butanol fractions of stem also showed good results against *Staphylococcus aureus* and *Klebsiella pneumonia* (Table 4).

Table 1. Phytochemical screening of *Justicia gendarussa*.

Species and part used	Phytochemicals	Extracts and sub Fractions					
		Crude	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Aqueous
***Justiciagendarussa* (roots)**	Alkaloids	+	–	+	+	–	–
	Flavonoids	+	–	–	–	+	+
	Terpenes	–	–	–	–	–	–
	Tannins	+	–	–	+	–	–
	Saponins	–	–	–	–	–	–
	Phenols	+	–	–	–	–	+
***Justiciagendarussa* (stem)**	Alkaloids	+	–	+	+	–	–
	Flavonoids	+	–	+	+	–	–
	Terpenes	–	–	–	–	–	–
	Tannins	+	–	–	+	–	–
	Saponins	–	–	–	–	–	–
	Phenols	+	–	–	+	+	–
***Justiciagendarussa* (leaves)**	Alkaloids	+	–	+	+	–	+
	Flavonoids	+	–	–	–	+	+
	Terpenes	–	–	–	–	–	–
	Tannins	+	–	–	+	–	–
	Saponins	–	–	–	–	–	–
	Phenols	+	–	+	+	–	–

(+) Presence, **(–)** Absence

Table 2. Phytochemical screening of *Justicia adhatoda*.

Species and part used	Phytochemicals	Extracts and sub Fractions					
		Crude	*n*-hexane	Chloroform	Ethyl Acetate	*n*-butanol	Aqueous
***Justicia adhatoda* (roots)**	Alkaloids	+	–	+	+	–	–
	Flavonoids	+	–	+	–	–	+
	Terpenes	+	+	–	–	–	–
	Tannins	–	–	–	–	–	–
	Saponins	–	–	–	–	–	–
	Phenols	+	–	–	–	+	+
***Justicia adhatoda* (stem)**	Alkaloids	+	–	+	–	-	+
	Flavonoids	+	–	-	+	+	+
	Terpenes	+	+	+	–	–	–
	Tannins	–	–	–	–	–	–
	Saponins	–	–	–	–	–	–
	Phenols	+	–	–	+	+	–
***Justicia adhatoda* (leaves)**	Alkaloids	+	–	+	–	–	+
	Flavonoids	+	–	+	+	–	–
	Terpenes	+	+	+	–	–	–
	Tannins	–	–	–	–	–	–
	Saponins	–	–	–	–	–	–
	Phenols	+	–	+	+	–	–

(+) Presence, (-) Absence

Antifungal activities: *In vitro* crude extracts and other fractions of both plants were testedagainst four fungal strains i.e. *Aspergillus niger, Aspergillus flavus, Fusarium solani and Aspergillus fumigates*; *and* compared with the standard antibiotic Clotrimazole (2µg/µL) (Tables 5 & 6). The crude extract, chloroform fraction, ethyl acetate fraction and aqueous fraction of *J. gendarussa* root showed positive results against *A. niger.* All fractions of *J. gendarussa* stem were active except *n*-butanol fraction and*n*-hexane fraction. Crude, chloroform and ethyl acetate fractions of leaves extracts were active while *n*-butanol, *n*-hexane and aqueous fractions were inactive against *A. niger.* Similarly *n*-hexane and n-butanol fractions of *Justicia adhatoda* showed positive results. Chloroform, Ethyl acetate and *n*-hexane fractions of *J. gendarussa* showed positive result against *Aflavuswhile Ethyl acetate fraction* of *Justicia adhatoda* was active against *A. flavus.* In case of *F. solani,* crude and chloroform fractions of *J. gendarussa* and *Justicia adhatoda* were active. Against *A. fumigates,* crude extract and chloroform fraction of *J. gendarussa* root were found active. While in case of *J. adhatoda* crude extract, chloroform fraction and ethyl acetate fractions of stem were found active against the tested fungal strains.

Table 3. Antibacterial activities of *Justiciagendarussa*.

Plant parts	Micro-Organisms	Inhibition zone (mm) *Justiciagendarussa*													
		Standards		Crude		*n*-hexane		Chloroform		Ethyl acetate		*n*-butanol		Aqueous	
		Amx*	**Lev	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2
Root	*Enterococcus faecalis*	–	29	20	24	–	–	21	25	20	22	18	20	–	–
	Salmonella typhi	–	26	20	22	–	–	19	21	15	16	16	18	12	15
	Proteus mirabilis	–	22	14	15	–	–	19	20	16	18	16	18	–	–
	Staphylococcus aureus	–	29	24	26	–	–	26	27	22	23	14	16	10	12
	Escherichia coli	–	22	18	20	–	–	18	19	16	18	16	18	18	20
	Klebsiella pneumoniae	–	28	20	21	–	–	18	21	14	18	–	–	–	–
Stem	*Enterococcus faecalis*	–	29	18	20	–	–	22	26	18	20	12	14	–	–
	Salmonella typhi	–	26	22	23	–	–	18	20	16	19	–	–	–	–
	Proteus mirabilis	–	22	16	17	–	–	20	22	18	20	–	–	–	–
	Staphylococcus aureus	–	29	25	27	–	–	23	24	18	20	–	–	–	–
	Escherichia coli	–	22	12	14	–	–	14	16	–	–	–	–	–	–
	Klebsiellapneumonia	–	28	22	23	–	–	19	21	18	20	–	–	–	–
Leaves	*Enterococcus faecalis*	–	29	18	20	–	–	22	26	18	20	12	14	16	18
	Salmonella typhi	–	26	22	23	–	–	18	20	16	19	18	20	14	16
	Proteus mirabilis	–	22	_	_	–	–	–	–	–	–	–	–	–	–
	Staphylococcus aureus	–	29	25	27	–	–	23	24	–	–	20	22	19	20
	Escherichia coli	–	22	_	_	–	–	–	–	–	–	–	–	–	–
	Klebsiella pneumonia	–	28	22	23	–	–	19	21	18	20	18	21	18	20

Table 4. Antibacterial activities of*Justicia adhatoda*.

Plant parts	Micro-Organisms	Inhibition zone (mm) *Justiciaadhatoda*													
		Standards		Crude		*n*-hexane		Chloroform		Ethyl acetate		*n*-butanol		Aqueous	
		Amx*	**Lev	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2
Root	*Enterococcus faecalis*	Amx*	**Lev	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2
	Salmonella typhi	–	29	22	24	–	–	24	26	18	20	–	–	–	–
	Proteus mirabilis	–	26	26	28	–	–	23	25	–	–	–	–	14	16
	Staphylococcus aureus	–	22	16	18	–	–	16	18	–	–	–	–	_	_
	Escherichia coli	–	29	26	28	–	–	24	26	20	22	14	16	16	18
	Klebsiella pneumoniae	–	22	20	22	–	–	18	20	16	18	–	–	14	16
Stem	*Enterococcus faecalis*	Amx*	**Lev	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2
	Salmonella typhi	–	29	_	_	–	–	–	–	–	–	–	–	–	–
	Proteus mirabilis	–	26	_	_	–	–	–	–	–	–	–	–	–	–
	Staphylococcus aureus	–	22	18	20	–	–	20	22	22	24	10	12	12	14
	Escherichia coli	–	29	24	26	–	–	26	28	28	30	14	16	24	26
	Klebsiellapneumonia	–	22	18	20	–	–	16	18	20	21	_	_	14	16
Leaves	*Enterococcus faecalis*	Amx*	**Lev	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2
	Salmonella typhi	–	29	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus mirabilis	–	26	–	–	–	–	–	–	–	–	–	–	–	–
	Staphylococcus aureus	–	22	10	11	12	13	12	14	_	_	10	12	18	20
	Escherichia coli	–	29	12	14	–	–	14	16	14	16	–	–	–	–
	Klebsiellapneumonia	–	22	9	10	–	–	–	–	10	12	–	–	12	13

Table 5. Antifungal activities of *Justicia gendarussa*.

Species and part used	Extracts and sub fractions	Fungal strains			
		A. niger	*A. flavus*	*F. solani*	*A. fumigatus*
***Justicia gendarussa* (Root)**	Crude	+	+	+	+
	n-hexane	–	+	–	–
	Chloroform	+	–	+	+
	Ethyl Acetate	+	+	–	–
	n-butanol	–	+	–	–
	Aqueous	+	–	–	–
***Justicia gendarussa* (Stem)**	Crude	+	+	+	+
	n-hexane	–	–	+	–
	Chloroform	+	+	+	–
	Ethyl Acetate	+	+	–	+
	n-butanol	–	–	–	–
***Justicia gendarussa* (Leaves)**	Crude	+	+	+	+
	n-hexane	–	–	–	–
	Chloroform	+	+	–	–
	Ethyl Acetate	+	+	–	+
	n-butanol	–	+	+	+
Standard drug Clotrimazole* (control)		+	+	+	+

* = Positive control

Table 6. Antifungal activities of *Justicia adhatoda*.

Species and part used	Extracts and sub fractions	Fungal strains			
		A. niger	***A. flavus***	***F. solani***	***A. fumigatus***
***Justicia adhatoda* (root)**	Crude	+	+	+	+
	n-hexane	+	−	+	−
	Chloroform	−	−	+	+
	Ethyl Acetate	−	+	−	+
	n-butanol	+	−	−	−
	Aqueous	+	−	+	−
***Justicia adhatoda* (stem)**	Crude	+	+	+	+
	n-hexane	+	−	+	−
	Chloroform	−	−	+	−
	Ethyl Acetate	−	+	−	+
	n-butanol	+	−	+	−
***Justicia adhatoda* (leaves)**	Crude	+	+	+	+
	n-hexane	+	−	−	−
	Chloroform	−	−	−	+
	Ethyl Acetate	−	+	+	−
	n-butanol	+	−	−	−
Standard drug Clotrimazole* (control)		+	+	+	+

* = Positive control

Conclusion

The present study demonstrates the phytochemical assessment of *Justicia gendarussa* and *Justicia adhatoda*. These phytochemicals seems to be responsible for diverse biological assays of the plant extracts. The current study report antimicrobial activities of *J. gendarussa* and *J. adhatoda* for the first time. Our results showed that both the plant species shows good potential against the tested antimicrobialessays. Based on our results it is recommended that the crude extracts and different solvent soluble fractions of our selected plants can be used for therapeutic purposes and also would escort to synthesize safe herbal drugs with least side effects.

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