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Phytochemical screening and evaluation of antioxidant, antimicrobial, cytotoxic and dermal irritant activities of *Ranunculus laetus* extracts

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ABSTRACT

In present study, different leaves, roots and floral extracts i.e. methanol, chloroform, and n-hexane of *Ranunculus laetus* (*R. laetus*) were tested to find out their bioactivities. The yield obtained from the methanolic extracts was 15.12%, 20.5% and 31.3%, while that of chloroform extracts 4.15%, 16.15% and 29.6% and that of n-hexane extract 4.52%, 10.55% and 31.8%. The established protocols were carried out with slight modifications for phytochemical and pharmacological activities. Phytochemical screening revealed the presence of carbohydrates, glycosides, cardiac glycosides, saponins, flavonoids, cumarins, steroids, proteins, and amino acids, fixed fats and oils in all fractions. Floral extracts were found to contain highest total phenolic contents (64 to 73 mg GAE/g), whereas leaves n-hexane fraction exhibited maximum DPPH potential (70 %). All the extracts showed promising antimicrobial activity in terms of zone of inhibition against selected bacterial strains, both Gram positive and Gram negative i.e. from 1.33 mm to 25.66 mm and against fungal strains i.e. from 1.33 mm to 24.33 mm. These findings suggest that the different parts of *R. laetus* exhibiting different biological activities and could be used as a promising source of novel drugs.

Keywords: *Ranunculus laetus*, phytochemical, antioxidant, antimicrobial, cytotoxicity, dermal irritant.

INTRODUCTION

The medicinal plants have been the primary source of therapeutic agents for health care since early ages and are still in practice in modern times. With recent developments in drug discovery and invention of modern medicines, the options for medical treatments have been increased but different pathogens have a common tendency to develop resistance against general medicines and intrusive medicines (Allen *et al.* 2010). Plants produce secondary metabolites as a part of their defense mechanism. A continuous research is underway to find out novel compounds from the plants, exhibiting the unique mode of action effective against drug-resistant pathogens.

The leaves and flowers of *R. laetus* belonging to family *Ranunculaceae* have therapeutically potential against tympany, conjunctivitis and diseases of eye. Related species of genus *Ranunculus* have variety of ethno-pharmacological uses such as *R. arvensis* is used to treat gout, asthma and intermittent fever (Khare 2008), *R. bulbosus.* is effective in gout, arthritis, neuralgia pains, antispasmodic, diaphoretic and rubefacient (Leporatti and Ghedira 2009), *R. chinensis* is used for the treatment of diarrhea and parasites (Shen *et al.* 2010), *R. diffusus* is used against rheumatism (Paulsamy*et al.* 2007), *R. hirtellus* is used as vermifacient and anthelmintic (Uniyal *et al.* 2006), *R. muricatus* is

Author's Contribution: Z.M, Collected data, performed practical and wrote manuscript; **F.S.,** Supervised and proof read manuscript; **S.A,** Analyzed data statistically; **T.J, P**erformed antimicrobial activity; **M.A, C**ollected data, did antioxidant activity and wrote manuscript; **I.W,** Performed dermal irritant activities; **M.A,** Performed Cytotoxic activities; **U.F.G**, Read manuscript

valuable for tumor, plague and abscess treatment (Hina *et al.* 2011), *R. repens* is antihemorrhagic (Mantle *et al.* 2000), *R. sceleratus* enhances blood circulation, reduces cold, swelling and also effective in internal abscesses, malaria, snake or scorpion venom and acute hepatitis (Mei *et al.* 2012) and *R. aqualitis* is indicated against intermittent fever, asthma and rheumatism. Some other members of this family are also used as a remedy for arthritis, neuralgia and sciatica in the form of ointments, liniments and poultice (Nelly *et al.* 2008).

Some known compounds have already been isolated from the chloroform extract of *R. laetus* including β-amyrin, jacedin-5-O-β-D-glucoside, 6,7-dimethoxycoumarin, centaurein, β-sitosterol-3-O-β-D glucoside and jacein (Hussain *et al.* 2009). Four of these compounds were found to be antibacterial in pure form. Despite immense medicinal potential in ethnomedicine, *R. laetus* has not yet been investigated for the isolation of antifungal, antioxidant, cytotoxic and dermal irritant constituents. Therefore in the present study leaves, roots and flowers of *R. laetus* were extracted with n-hexane, chloroform and methanol to screen out their antimicrobial, antioxidant, cytotoxic and dermal irritant effects.

MATERIALS AND METHODS

Chemicals and instruments

Analytical grade solvents and chemicals were used for all purposes, except if mentioned otherwise. The study was conducted as per approved protocol by the Animal Ethics Committee, Department of Pharmacy, The University of Lahore, Lahore, Pakistan in accordance with "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985). The bacterial and fungal strains were collected from PCSIR (Pakistan council of scientific and industrial research) Lahore, Pakistan. Four Gram-positive bacterial strains such as *Staphylococcus aureus* (ATCC 12600), *Bacillus subtilis* (ATCC 11774), *Micrococcus luteus* (ATCC 10240) and *Salmonella typhi* (ATCC 14028) and four Gram-negative strains: *Klebsiella pneumoniae* (ATCC 13882), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739) and *Shigella flexneri* (ATCC 700930) were selected for this study. The antifungal activity was conducted against two human pathogenic fungal strains, i.e., *Fusarium solani* (ATCC 11712) and *Candida albicans* (ATCC10231).

Plant material

About 8 kg of *R. laetus* plants were collected from the forest of Murree ($33^{o}54'$ N; $73^{o}23'$ E; 2291 m altitude), Punjab Pakistan in April, 2013 and authenticated and voucher specimen (voucher number 0274) was deposited in the herbarium of The University of Lahore, Lahore 54600, Pakistan.

Extraction method

Roots, leaves and flowers were separated, thoroughly washed with water at ambient temperature, subjected to air drying in shadow, pulverized, sieved through 80 mesh sieves and stored in the sterile amber glass bottle. Each ground part of the plant was separately extracted with 700 ml of a solvent (n-hexane, chloroform or methanol) using Soxhlet extractor until the solvent in thimble chamber appeared nearly colorless (max. 72 hrs). Extracts were filtered and concentrated on the rotary evaporator at 35^{o}C and subjected to freeze-drying to get extracts.

Phytochemical and pharmacological screening

The dried plant extracts were subjected to phytochemical, antioxidant, antibacterial, antifungal, cytotoxicity and irritancy screening using standard methods with some modifications as described below.

Phytochemical screening

Different qualitative phytochemical tests were performed on leaf, root and flower extracts of *R. laetus* for the presence of various phyto-constituents (Waheed *et al.* 2014; Trease and Evans 1997).

Antioxidant assays

Already reported protocols were followed to explore antioxidant potential of plant extracts by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Ikram *et al.* 2009), ferric reducing antioxidant power (FRAP) and total phenolic content (TPC) assay (Benzie and Strain 1996).

Antimicrobial assay

The antimicrobial activity was carried out by agar well diffusion method. Different dilutions of extracts (0.1, 1, 10 and 100 mg/ml in normal saline) were tested while ciprofloxacin and methicillin (20 µl, 1 mg/ml in DMSO) were used as antimicrobial standards and clotrimazole (20 µl, 1 mg/ml in DMSO) was used as antifungal standard (Naz and Bano 2012).

Brine shrimp lethality assay

Cytotoxicity of extracts was tested at different concentrations (10, 100 and 1000 µg/ml) along with umbelliferone (5, 50 and 500 µg/ml) as a standard. The experiment was repeated in triplicate, mean percentage mortality was calculated and median lethal concentration (LC_{50}) was determined from log of extract concentration verses mean percentage mortality curve (Michael *et al.* 1956).

Dermal irritant activity

A group of six rabbits was used to inspect irritant activity of each extract. Acetone solution of extract (10 mg/ml) was applied on the inner surface of rabbit's one ear at different dose levels (20, 40, 80 and 120 µl) and other ear was used as negative control. For allergic response ears were observed after equal intervals of 30 minutes for 3 hrs. and later on after 24 and 48 hrs. The number of ears showing significant inflammation of the major blood vessels was noted to calculate irritant dose in 50% of the test animals (ID_{50}) (Saeed *et al.* 2013).

RESULTS AND DISCUSSION

Extraction and phytochemical screening

Dry powders of leaf, root and flower (85 g, 20 g and 8 g respectively) were separately extracted with n-hexane, chloroform and methanol to isolate nonpolar, partially polar and polar constituents of *R. laetus*. After evaluating the extract yields from different parts of the plants (Table 1), the phytochemical studies were undertaken to identify the secondary metabolites in the extracts using standard reported identification methods and results are provided in Table 2. The phytochemical analysis of various parts of *R. laetus* indicated that phenolics, tannins, saponins, flavonoids, betacyanin, cardiac glycosides, coumarin, steroids, phytosterols, carbohydrates and proteins were present while alkaloids, terpenoids, anthraquinones and quinones were absent in all parts of the plant.

Table I: Percentage yields as per dry plant masses of *R. laetus* extracts from n-hexane, chloroform and methanol.

Plant section	Weight (gm)	Extract yield					
		n-Hexane		Chloroform		Methanol	
		Extract weight (gm)	Dry mass (%)	Extract weight (gm)	Dry mass (%)	Extract weight (gm)	Dry mass (%)
Leaf	85	3.85	4.52	3.54	4.15	12.85	15.12
Root	20	2.11	10.55	3.22	16.15	4.1	20.5
Flower	8	2.52	31.8	2.37	29.6	2.52	31.3

Table II: Phytochemical analysis of *R. laetus* extracts from n-hexane (C_6H_{14}), chloroform ($CHCl_3$) and methanol (CH_3OH).

	Leaf			Root			Flower		
Solvent	C_6H_{14}	CHC	CH_3O	C_6H	$CHCl_3$	CH_3	C_6H_{14}	$CHCl_3$	CH_3
Alkaloids									
Mayer's	-	-	-	-	-	-	-	-	-
Wagner's	-	-	-	-	-	-	-	-	-
Hager's	-	-	-	-	-	-	-	-	-
Carbohydrates									
Molish's	+++	++	+++	+++	+++	+++	-	+	++
Fehling's	+	+	+	++	++	++	-	-	-
Barfoed's	-	-	-	-	-	-	-	-	-
Benedict's	+++	+++	+++	-	-	++	-	+	++
Glycosides									
Borntrager'	-	-	-	-	-	-	-	-	-
Legal's test	++	++	++	++	++	++	++	++	++
Cardiac	+++	+++	+++	+	+++	+++	-	+	+
Protein and amino acids									
Biuret test	+++	-	++	++	++	++	+	+	++
Ninhydrin	++	++	++	+	-	+	-	-	-
Fixed oils and fats									
Spot test	+	+	+	++	++	++	++	++	++
Saponificati	+++	+++	+++	++	++	++	++	++	++
Phenolics and tannins									
Ferric	-	-	++	-	-	-	-	-	-
Gelatin test	-	-	+	-	-	-	-	-	-
Lead	-	-	++	-	-	-	-	-	-
Gums and	++	++	++	-	-	-	-	-	-
Saponin	++	++	++	++	++	++	++	++	++
Flavonoid	+	+++	+++	+	+++	+++	+	+++	+++
Anthocyan	-	-	-	-	-	-	-	-	-
Betacyani	++	+++	+++	+	+++	+++	+	+++	+++
Terpenoid	-	-	-	-	-	-	-	-	-
Coumarin	+	+++	+++	+	+++	+++	+	+++	+++
Acids	-	-	-	-	-	-	-	-	-
Steroids	+	+++	+++	+++	+++	+++	+++	+++	+++
Phlobatan	-	-	-	-	-	-	-	-	-
Anthraqui	-	-	-	-	-	-	-	-	-
Quinones	-	-	-	-	-	-	-	-	-
Absent = -, Weakly present = +, Moderately present = ++, Strongly present =+++									

Antioxidant assays

Till date, no antioxidant assays have been performed on the extract of *R. laetus*. DPPH, FRAP and total phenolic contents (TPC) are among potent assays to investigate antioxidant potential. The results of DPPH, FRAP and TPC for n-hexane, chloroform and methanol extracts of different parts *R. laetus* were presented in Figure 1. For extracts and standards, DPPH activity was determined in terms of percentage radial scavenged activity (%RSA) at a final concentration of 20 µg/ml. All extracts were found promisingly active as compared to the standards vitamin E and vitamin C. In comparison, vitamin E was found 2.1 to 1.2 folds more active while vitamin C was found 1.8 to 1.03 folds more active than extracts. In sequence, n-hexane extract of leaf was the most active against oxidants whereas the root extract in the same solvent had minimum activity. According to the literature DPPH activity is performed on whole plant n-hexane, ethyl acetate, methanol and water extracts of *R. marginatus var. trachycarpus* and *R. sprunerianus* at a final concentration of 55.5 µg/ml (20). n-Hexane extracts of *R. marginatus var. trachycarpus* and *R. sprunerianus* were reported least active (10.50 and 27.60% respectively) and methanol extracts were most active (76.58 and 85.34% respectively). DPPH activity of *R. laetus* has values in the range of 40.46 to 69.61% at a concentration of 20 µg/ml and it could be regarded as more active than *R. marginatus* and *R. sprunerianus*.

The trends in results of FRAP and TPC assays were quite in agreement showing methanolic extract from flower was the most active and extract of leaf from the same solvent had minimum activity. It is reported that different extracts of *R. marginatus var. trachycarpus* and *R. sprunerianus* have TPC in the range of 122.8 to 694 mg GAE/g which is quite high as compared to *R. laetus* (25 to 67 mg GAE/g) (Kaya *et al.* 2010).

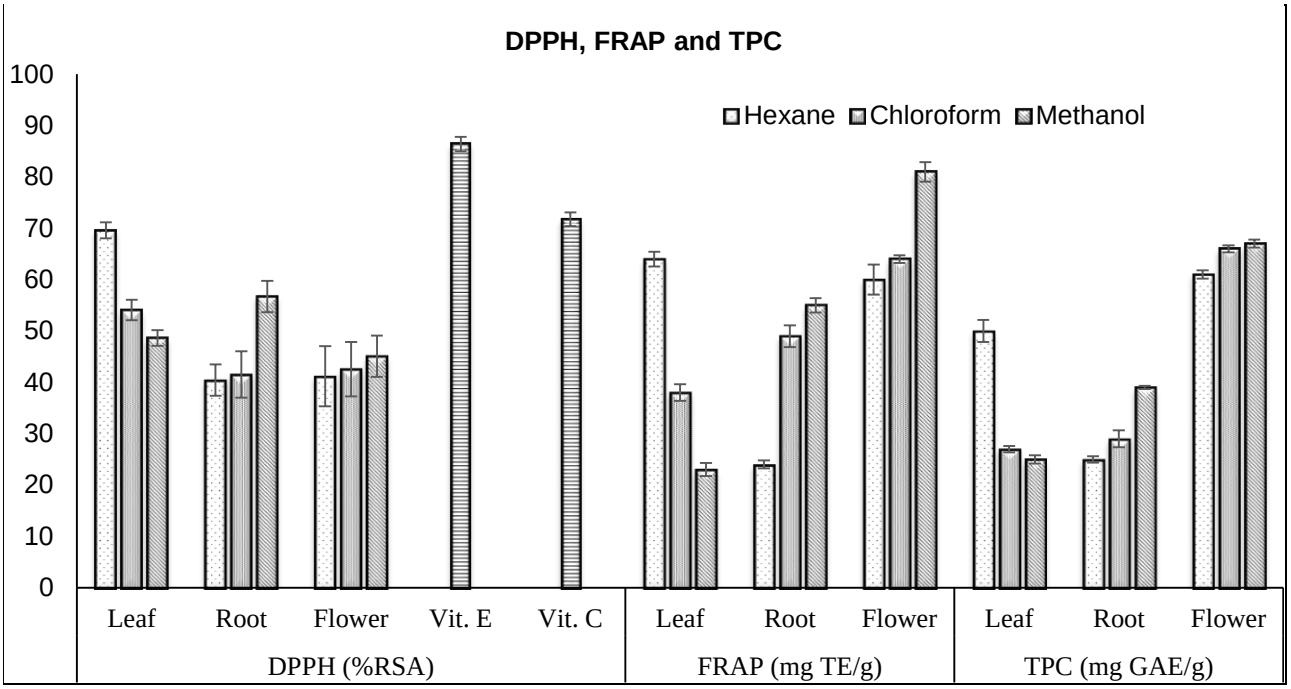

Fig. 1: DPPH, FRAP and TPC of n-hexane, chloroform and methanol extracts of leaf, root and flower of *R. laetus.*

Shahid *et al* (2015) reported DPPH, FRAP and TPC assays on n-hexane, chloroform, ethyl acetate, n-butanol and water-soluble fractions of whole plant extract of *R. sceleratus*. According to their results, n-hexane and ethyl acetate fractions had minimum and maximum TPC (23.5 and 97.1 TE µM/mL respectively) and FRAP activities (34 and 238.5 TE µM/mL respectively) (Shahid *et al.* 2015). These trends are in harmony with root and flower extracts of *R. laetus* but contradictory to leaf extracts of *R. laetus*. The total phenolic contents were maximum in all solvent fractions from the flower of the plant.

Antimicrobial assay

Four Gram-positive, four Gram-negative and two fungal strains were employed to check the antimicrobial potential of *R. laetus* extracts. Agar well diffusion method was selected and different concentrations (0.1, 1, 10 and 100 mg/ml) of extracts were used to find zones of inhibition against standard drugs at 1 mg/ml. All extracts presented zones of inhibition between 16 and 26 mm at 100 mg/ml against selected bacterial strains except chloroform leaf extract against *S. typhi*, n-hexane root extract against *S. aureus* and chloroform flower extract against *S. typhi* and *K. pneumoniae*, which were not significantly antibacterial up to 100 mg/ml. Zones of methicillin and ciprofloxacin were between 22 and 27 mm against selected bacterial strains at 1 mg/ml.

The results on the antibacterial activity of *R. laetus* recorded (Table 3) were found similar to those of in the present study conducted by Hussain et al. (2009) on the same plant against eight pathogenic bacterial strains including three different, i.e., *K. pneumoniae, M. luteus and S. typhi*. The antimicrobial activity of *R. laetus* could be due to the presence of phenolics, tannins, saponins, flavonoids, Coumarin and phytosterols.

R. laetus extracts were further tested for antifungal activity against two human pathogenic fungal strains, *C. albicans* and *F. solani,* depicting promising results as compared to standard clotrimazole (Table 3). Zones of inhibition of all extracts were found between 18 and 25 mm at 100 mg/ml concentration against both of the selected fungal strains, except n-hexane extracts of leaf and root which did not show zones at tested concentrations. Clotrimazole depicted nearly 46 mm zones against both of the strains at 1 mg/ml. n-Hexane extract of flower was found most active particularly against *C. albicans*. Chloroform and methanol extracts of all parts represented comparatively good antifungal activity at all tested dose levels against both tested fungal strains. Antifungal activity of other *Ranunculus* species (*R. sceleratus* L., *R. asiaticus* and *R. arvensis* L.) studied against variety of fungal strains as reported in literature was found in harmony with results of the present study (Misra and Dixit 1978; Qasem and Abu-Blan 1995 and Bhatti *et al.* 2015).

Brine shrimp lethality assay

Brine shrimp lethality assay, a key preliminary investigation tool for the assessment of cytotoxicity, has already been reported for some other species of *Ranunculus* such as *R. ternata* (Yin *et al.* 2008) and *R. sieboldii* (Pan *et al.* 2004), but the present work is first account of this activity for *R. laetus*. The results recorded here are quite in agreement with those of other studies. Comparison of leaf, root and flower extracts had been outlined in Figure 2. All three n-hexane extracts of leaf, root and flower were most cytotoxic fractions with LC_{50} values of 159.8, 215.5 and 252.2 µg/ml respectively. These promising activities of crude extracts established the potential of further investigation for the isolation of cytotoxic compounds. Methanol extracts were among the least cytotoxic fractions, which is in agreement with previous studies on methanol extracts of *R. arvensis*, *R. occidentalis* and *R. repens* with LC_{50} greater than 1000 µg/ml (Karchesy *et al.* 2016).

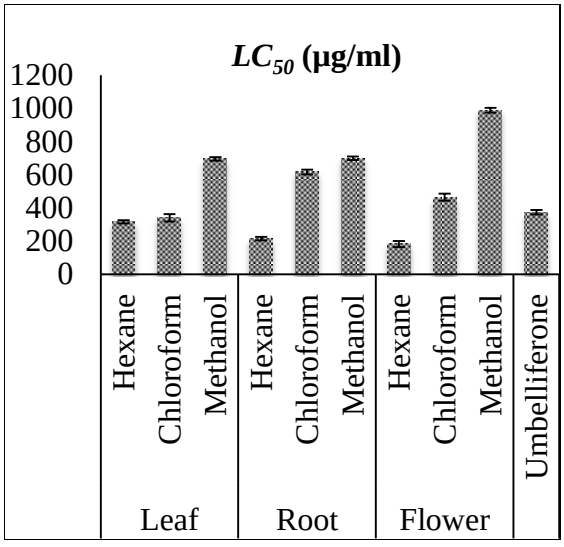

Fig. 2: Cytotoxic activity of *R. laetus* leaf, root and flower extracts from n-hexane, chloroform and methanol using brine shrimps (*Artemia nauplii*).

Dermal irritant activity

No acute or chronic irritant activity was observed at all applied dose levels (20, 40, 80 and 120µl at a concentration of 10 mg/ml). This accounts for the presence of non-irritant compounds in *R. laetus* and validates its safe use for dermal applications in the form of ointments, liniments and poultice. There are contradictory evidences about the irritant activity of *Ranunculus* species. Some species of this family can cause serious skin inflammation while some species are used in the form of ointment. Poultices of *R. arvensis* and *R. constatinopolitanus* are reported to cause phytodermatitis analogues to burn like injury (Akbulut *et al.* 2011 and Ceyhan *et al.* 2010).

CONCLUSION

Phytochemical investigations suggest the presence of flavonoids, steroids, cumarins and saponins in *R. laetus*. These metabolites are usually bioactive and could be responsible for its biological activities. Antimicrobial potential of *R. laetus* along with its promising antioxidant, and cytotoxic activities make it a very promising candidate for further investigations to find out future drugs for studied activities. According to brine shrimp assay n-hexane extracts from leaf, root and flower are suitable for isolation of cytotoxic constituents. Non-irritant effect of plant also supports its safe use.

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Table III: Antibacterial and antifungal activities of *R. laetus* extracts in different solvents

Part of *R. laetus*	Extraction Solvent	Conc. (mg/ml)	Bacterial strains								Fungal strains	
			Gram positive				Gram negative					
			S. aureus	*B. subtilis*	*M. luteus*	*S. typhi*	*K. pneu-moniae*	*P. aerug-inosa*	*E. coli*	*S. flexneri*	*C. albicans*	*F. solani*
Leaf												
	n-Hexane	0.1	2.66±0.30	2.86±0.30	1.89±0.36	1.98±0.26	1.33±0.36	2.66±0.30	5.58±0.30	-	-	-
		1	8.33±0.32	8.56±0.33	4.53±0.30	6.96±0.35	7.51±0.41	8.66±0.35	14.33±0.39	3.66±0.36	-	-
		10	13.66±0.35	14.83±0.27	12.08±0.50	13.33±0.53	12.46±0.50	14.46±0.40	19.07±0.47	11.66±0.36	-	-
		100	19.66±0.30	19.77±0.35	17.82±0.34	20.33±0.44	19.43±0.36	19.61±0.55	24.66±0.39	17.66±0.30	-	-
	Chloroform	0.1	3.66±0.35	1.33±0.36	1.33±0.27	-	2.39±0.30	2.66±0.30	3.60±0.35	1.53±0.13	1.79±0.22	2.63±0.30
		1	9.66±0.36	7.33±0.30	7.07±0.35	-	7.78±0.33	9.66±0.36	10.76±0.29	6.37±0.28	6.33±0.38	9.29±0.26
		10	15.33±0.34	12.33±0.31	12.95±0.40	-	15.75±0.30	15.66±0.30	15.50±0.42	11.59±0.41	12.48±0.30	14.77±0.44
		100	20.66±0.30	18.33±0.31	17.66±0.30	2.01±0.21	23.26±0.34	21.66±0.34	22.91±0.36	16.66±0.36	18.29±0.34	19.91±0.38
	Methanol	0.1	5.33±0.34	-	2.93±0.30	1.06±0.16	1.56±0.25	-	2.58±0.27	-	1.33±0.29	4.16±0.21
		1	12.66±0.36	3.93±0.53	9.73±0.38	6.36±0.39	9.33±0.36	3.74±0.36	10.66±0.30	4.93±0.36	7.56±0.20	10.78±0.40
		10	18.33±0.32	11.35±0.39	14.09±0.51	12.76±0.30	15.53±0.34	7.37±0.30	17.56±0.36	11.06±0.42	14.33±0.36	15.61±0.26
		100	24.23±0.30	17.33±0.47	19.66±0.30	18.66±0.34	20.61±0.30	16.26±0.48	24.61±0.39	17.66±0.37	19.06±0.49	22.08±0.39
Root												
	n-Hexane	0.1	-	2.56±0.29	2.81±0.30	1.66±0.25	2.56±0.30	4.61±0.30	3.76±0.32	-	-	-
		1	-	7.61±0.25	9.33±0.36	10.53±0.34	9.83±0.36	9.66±0.36	10.66±0.30	5.33±0.33	-	-
		10	-	14.33±0.36	14.76±0.30	15.29±0.37	14.76±0.30	15.71±0.55	15.96±0.36	12.66±0.30	-	-
		100	-	20.66±0.30	19.28±0.54	22.66±0.42	19.18±0.51	20.06±0.28	26.66±0.36	19.33±0.36	-	-
	Chloroform	0.1	5.53±0.15	1.23±0.22	1.88±0.15	2.43±0.27	2.39±0.18	3.76±0.35	2.16±0.16	-	4.76±0.10	3.05±0.16
		1	10.88±0.31	7.38±0.30	7.26±0.44	7.26±0.30	7.74±0.32	9.60±0.22	12.27±0.35	10.73±0.21	10.16±0.43	7.56±0.29
		10	15.86±0.43	12.33±0.52	13.86±0.29	15.38±0.42	14.53±0.50	14.07±0.47	19.81±0.28	17.44±0.30	15.75±0.30	12.16±0.30
		100	22.66±0.36	19.33±0.36	17.66±0.30	22.66±0.36	19.66±0.30	20.66±0.30	24.66±0.44	19.33±0.36	22.66±0.36	19.33±0.36
	Methanol	0.1	4.93±0.15	2.56±0.18	3.89±0.27	1.13±0.36	3.33±0.36	4.16±0.25	5.86±0.20	-	2.23±0.19	2.56±0.22
		1	9.27±0.52	9.33±0.36	9.33±0.36	6.66±0.36	7.33±0.30	8.66±0.35	12.66±0.36	5.33±0.33	7.33±0.30	8.71±0.35
		10	16.76±0.34	14.93±0.41	13.16±0.29	13.80±0.31	15.92±0.39	15.66±0.31	15.66±0.36	14.83±0.36	12.66±0.28	14.35±0.44
		100	22.33±0.36	19.66±0.30	19.66±0.30	20.33±0.34	20.66±0.30	20.66±0.30	25.66±0.36	20.33±0.34	19.33±0.36	19.66±0.30

Flower	n-Hexane	0.1	5.66±0.18	-	2.73±0.26	3.56±0.35	1.93±0.20	3.76±0.27	5.29±0.14	-	4.34±0.19	2.93±0.10
		1	12.26±0.37	6.33±0.28	7.86±0.41	9.16±0.29	7.26±0.27	8.56±0.38	10.16±0.22	4.86±0.29	12.46±0.36	6.86±0.28
		10	16.96±0.36	10.43±0.32	14.33±0.30	15.92±0.30	12.16±0.19	14.44±0.29	20.83±0.49	8.33±0.36	17.66±0.36	12.65±0.30
		100	23.66±0.34	20.66±0.30	20.66±0.30	22.66±0.36	19.33±0.36	20.66±0.30	24.66±0.25	19.66±0.30	24.33±0.32	19.33±0.49
	Chloroform	0.1	5.33±0.34	1.33±0.36	2.33±0.30	-	-	7.16±0.26	4.63±0.36	-	1.96±0.26	2.68±0.28
		1	12.26±0.46	3.93±0.26	9.03±0.29	-	-	13.46±0.35	10.16±0.30	5.02±0.27	7.16±0.31	6.96±0.25
		10	17.76±0.34	12.66±0.30	14.13±0.26	-	-	21.83±0.41	18.33±0.32	10.75±0.36	12.16±0.40	14.23±0.21
		100	25.66±0.36	20.66±0.30	21.66±0.34	-	-	24.66±0.39	25.66±0.36	17.33±0.30	19.33±0.36	19.66±0.30
	Methanol	0.1	7.66±0.36	1.33±0.33	1.53±0.36	4.66±0.30	2.66±0.30	3.63±0.32	5.13±0.34	-	3.66±0.35	1.66±0.36
		1	13.66±0.35	6.66±0.36	7.66±0.30	10.53±0.24	9.74±0.31	11.66±0.34	10.66±0.30	6.33±0.36	9.33±0.36	6.66±0.29
		10	19.33±0.32	11.66±0.41	15.66±0.30	18.66±0.22	14.83±0.39	17.33±0.26	19.16±0.37	11.96±0.56	14.76±0.28	11.96±0.30
		100	24.66±0.39	19.66±0.30	20.66±0.30	22.82±0.36	19.33±0.36	22.66±0.36	24.66±0.39	16.66±0.36	20.66±0.30	19.33±0.36
Meth.		1	23.66±0.30	26.66±0.30	25.33±0.30	23.33±0.31	20.66±0.31	24.33±0.32	20.66±0.31	22.33±0.31	NT	NT
Cipro.		1	26.33±0.30	28.33±0.30	24.33±0.31	24.33±0.31	20.33±0.31	24.66±0.40	22.33±0.30	22.66±0.31	NT	NT
Clot.		1	NT	NT	NT	NT	NT	NT	NT	NT	46.66±0.40	46.33±0.31

Conc. = Concentrations, - = No zone of inhibition, Meth. = Methicillin, Cipro. = Ciprofloxacin, Clot. = Clotrimazole and NT = not tested