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ORIGINAL ARTICLE

Antibacterial activity and phytochemical analysis of *Impatiens balsamina L.* under heavy metal (Nickel) stress

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ABSTRACT
Impatiens balsamina L, is a plant belonging to the family Balsamiaceae. It is an annual herb which originated in Southern Asia. The whole plant has been used as indigenous medicine for the treatments of rheumatism, warts, snake bite and finger nail inflammation. This plant species is known as an ornamental and as well as a medicinal plant. Plants are known to be affected by various stresses, out of which heavy metals are reported to cause detrimental effects. In the present study, the effect of Nickel (Ni) on Impatiens balsamina L. (leaf and flower) phytochemical constituents (alkaloids, glycosides, saponins, sterols, phenol, flavonoids, tannins, quinones, terpenoid and anthocyanin) and the potential of its various solvent extracts (aqueous, hexane and methanol) were tested towards antibacterial activity (zone of inhibition (ZOI)). In methanolic extracts, except saponins all the phytochemical constituents were detected in the 'Ni' treated plant in comparison with only two in its control (alkaloid and anthocyanin). The methanolic extracts were shown to have highest amount of antibacterial activity in comparison with other extracts, especially the 'Ni' treated methanolic extracts showed statistically significant ($p \leq 0.05$) increase or higher ZOI in comparison with controls and antibiotic. These heavy metal exposed plant extracts can be a source of novel metabolites that can be further explored for its therapeutic uses.
Keywords: *Impatiens balsamnia L.*, Nickel, Antibacterial activity, Phytochemicals

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INTRODUCTION

Impatiens balsamina **(***I. balsamnia)* is native to Asia. It belongs to the *Balsaminaceae* family and consists of more than 1000 genera but few are known for its medicinal therapeutic significance [1]. It is found tremendously in India and is widely used as a folklore medicine to treat burns, scalds, rheumatism, abscesses, tumours and other diseases [2-4] and it was also reported to act as an metic, cathartic and diuretic [5]. Most of its plant parts such as root, flower, seed, leaf and even the whole herb were reported to have medicinal properties (6). Several compounds have been isolated from *I. balsamnia*, including 1, 4-naphthoquinone, triterpenoids, Baccharane, Ursane etc. [7-8]. Its phytochemicals were shown to be non-nutritional in nature but are important for numerous human body functions [9]. In Ayurveda, *I. balsamina* is recognized as a rich source of metabolites with antibacterial activity, which has been traditionally used in many parts of Asian countries like India, China, and Korea, etc [10]. The plant is traditionally used to treat thorn or glass-puncture wounds, abscesses [11], scrofulosis, carbuncles, dysentery [12], rheumatism and aches, fractures, superficial infections, fingernail inflammation, tumour, difficult labour and puerperal pain [10]. It was also reported that tea made from dried plant was used to treat systemic bacterial and fungal infections [13-14]. Such antagonistic properties can be attributed to plant secondary metabolites [15]. As a part of its defence mechanism, plants were known to produce the secondary metabolites that were reported to have antimicrobial properties [16-18]. These plant metabolites were shown to be affected by heavy metals [19-20]. Agricultural activities (fertilizer and pesticide applications) were shown to be one of the major sources for ground water contamination by heavy metals [21]. These heavy metals such as Nickel (Ni) were reported to contaminate soil and ground water ecosystems

by anthropogenic activities, soil leaching and chemical weathering of minerals thereby affecting human health [22]. But Ni is an essential nutrient for higher plants involving many biochemical and physiological processes that are crucial for growth and development, such as nitrogen metabolism, iron absorption and in maintaining proper cellular status for redox reactions [23-25]. Currently, Ni contamination became a significant problem due to increase in mining, fossil fuel burning, urban and industrial waste and fertilizer & pesticide application [23-26]. It was reported that due to increased farming and industrial activity, high amounts of Nickel (Ni) including other heavy metals were detected in soils of South Gujarat region [27]. Remediation or utilization of such heavy metal polluted soils have become a challenge [28-29]. Studies have reported that the presence of heavy metals has improved the plant metabolite content. Khan.W.U.*et al*., [30] showed that when plant *Vinca rosea* was subjected to Ni stress, the biomass production, protein synthesis and proline content were reported in higher amounts. Manjunath *et al*., [31] showed that when plant *Eryngium foetidum* exposed to certain amount of Ni i.e., 50ppm the production of phenols, flavonoids and saponins was also increased in the leaves. There was an increase in the lycopene and anthocyanin content of *Calendula officinalis* under Ni stress [32]. Not only medicinal plants, vegetable plant species such as eggplant also showed an increased in antioxidant activity under Ni stress (33). I. balsamnia L is a medicinal and nutritional value herb with potent secondary metabolites, which has been grown as an ornamental plant and, was reported for heavy metal tolerance and for the remediation of the heavy metals [34, 35]. There have been few studies on the effects of heavy metals on plant species, including Ni, but to our knowledge, there have been even less investigations on Ni's impact on plant secondary metabolites. The aim of the present study is to investigate the effect of heavy metal i.e., Ni on the I. balsamnia (medicinal plant) for their use in horticulture as well as in medicine, phytoremediation and for improving the aesthetics of the environment. The present study was to carried out to investigate the qualitative phytochemical study and antimicrobial activity of different extracts of the *I. balsamnia* L. (leaf and flower) under Ni induced stress for their antimicrobial potential.

MATERIAL AND METHODS

Collection and preparation of plant material:

Seeds of *Impatiens balsamina* were collected from the botanical garden Waghai, Gujarat. Plant species was authenticated by Dr. M. N. Reddy, Head of Bioscience Department, VNSGU, Surat, Gujarat (PANDYA/CGBIBT/06/20-21/IMPATIENS). Seeds were sown into the pot containing Ni in the form of NiCl2 (Himedia, Mumbai) at the concentration of 100mg/Kg of D.W. into the soil. The treatments were designed according to Gopal *et al*., [36] with some modifications. Seeds were surface sterilized and ensured for no microbial contamination and latter sown into the soil [37]. Leaves and flowers were collected at the flowering stage and dried in a cool dry area for 10 days at room temperature. The setup of this experiment was part of a larger experimental design focusing on the study of microbial-plant interactions with respect to heavy metal stress.

Preparation of plant extract:

Powdered plant material (flower and leaf) was extracted with different solvents (Aqueous, Hexane and Methanol) using Soxhlet extraction. According to Alara O.R. *et al*., [38] with some modification, leaves of *I. balsamina* were weighed and the respective solvents were used in feed-to-solvent ratio (1:1 g/ml), 100 gm of plant material was suspended with 100 ml of respective solvents and was placed in the extraction apparatus. Then, the extract was filtered through a filter paper (Whatman no. 1) and concentrated to dryness using a rotary evaporator. The extracts were stored in a refrigerator at 4°C until further analysis.

Yield of Extracts:

The extracts were filtered using Whatman No. 1 filter paper, the filtered extracts were concentrated by a rotary evaporator, and the residual extracts were dried. The percentage yield was obtained using dry weight, from below equation [39].

%Yield of extract (g/100 g) = (W1 × 100) / W2

Where W1 is the weight of the extract residue after solvent removal and W2 is the weight of dried plant powder

Qualitative phytochemical analysis from prepared extract:

Phytochemical screening of the flower and leaf extracts of *I. balsamina* was carried out as per the following procedures.

1. Test for Alkaloid

Hager's test: 2mg of extract in a test tube. To this add few drops of Hager's reagent. Formation of yellow precipitate confirms the presence of alkaloids [40].

2. Test for Glycosides

2ml of plant extract treated with 1ml of glacial acetic acid and 5% ferric chloride. To these 3drops of Conc. Sulphuric acid was added. Presence of greenish blue color indicates the presence of glycosides [41].

3. Test for Saponins (Foam test)

A few drops of sodium bicarbonate solution was added to the 2ml extract solution. The mixture was shaken vigorously and left for 3minutes. The formation of honey comb like froth indicates the presence of Saponins [42].

4. Test for Sterols

Salkowski test: 2mg of dry extract was shaken with chloroform, to the chloroform layer sulfuric acid was added slowly by the side of the test tube. Formation of the red color indicate the presence of steroids [43].

5. Test for Phenol

Ferric chloride test: The extract was treated with aqueous 5% ferric chloride and observed for the formation of deep blue or black color [44].

6. Test for Flavonoids

Alkaline reagent test: 2ml of extract was treated with a few drops of 10% sodium hydroxide solution. Formation of intense yellow color, which become colorless on the addition of dil. hydrochloric acid, indicates the presence of flavonoids [45].

7. Test for Tannins

1ml of extract treated with 1 to 2 drops of ferric chloride solution with 1ml of distilled water. The extract solution was thoroughly mixed and left for few minutes until formation of a blue or green black coloration [46].

8. Test for Quinones

A small amount of extract was treated with Conc. HCl and observed for the formation of a yellow color precipitate [47].

9. Test for Terpenoid

In a test tube, 0.5gm of plant extract was added with 2ml of chloroform. Add concentrated sulphuric acid carefully to form a layer. Observe for the presence of reddish-brown color interface to show positive result for the presence of Terpenoids [48].

10. Test for Anthocyanin

2ml of plant extract treated with 1ml of 2N sodium hydroxide and heated for 5 minutes at 100°C. Formation of bluish green color indicates the presence of anthocyanin [49].

Test microorganisms:

Strains of bacteria such as *Escherichia coli* (MTCC 2127), *Staphylococcus aureus* (MTCC 7443), *Salmonella typhi* (MTCC 53648) and *Pseudomonas aeruginosa* (MTCC 2642) were procured from MTCC (Microbial Type Culture Collection), Chandigarh, India. The microorganisms were sub-cultured on a nutrient agar slant and incubated at 37°C for further use.

Antimicrobial activity (Agar well diffusion assay):

The antimicrobial activity of leaf and flower extracts were evaluated by agar well diffusion method (50-52). The bacterial strains were inoculated on a minimal agar plate (HiMedia, Mumbai, India). After inoculation of the bacterial strains, wells were punched using sterile cork borer. The wells were filled up with 200µl of plant extracts (4mg/ml) and DMSO. The extracts were dissolved in 20% DMSO (dimethyl sulfoxide) and then transferred into the well and the final concentration of DMSO was 0.1% (below 1% is not known to exert any toxic effect) [53].

Determination of MIC by Broth Dilution Technique:

Broth dilution technique was used to determine the Minimum Inhibitory Concentration (MIC) of the plant extracts against five bacterial strains [54-55]. The plant extracts were dissolved in DMSO and final DMSO was maintained at 1:1 with some modifications for concentration. The extract was added to each tube to keep the final concentration ranging from 31.25µg/ml to 1000µg/ml. The test bacterial suspension was added into each tube with sterile nutrient broth (1ml), to yield a bacterial density of 106 CFU/ml and the inoculated tubes were incubated at 37˚C for 24 h. Test tubes containing nutrient broth without plant extract and bacteria, followed by only test bacteria were kept as controls. After incubation, 50µl of 0.2mg/ml *p*-iodonitrotetrazolium violet (INT) was added in each tube to indicate the bacterial growth. The tubes were again incubated for 30min at 37˚C. Development of pink color in the tube (due to reduction of dye) indicated the bacterial growth, whereas tubes without color indicated no active bacterial growth. The lowest concentration at which no bacterial growth was observed (as indicated by color) corresponded to the minimum inhibitory concentration (MIC). All the assays were performed in triplicates [56].

Heavy metal analysis:
Nickel content in the leaves and flower was estimated by AAS from Environment care laboratory, Bardoli, Gujarat, India.

Statistical analysis:
The antimicrobial activity including MIC was setup in triplicates. The bar diagrams were plotted using Prism 5.0 version. The statistical significance ($p \leq 0.05$) was measured by SPSS (16.0 Version). The ZOI of the other comparisons were given as supplementary information.

RESULTS AND DISCUSSION

Table 1: Percentage Yield of *I. balsamina (Leaf and Flower) using* Soxhlet extraction with different solvents

Plant	Part used	% Yield (w/v)		
		Hexane	Aqueous	Methanol
I. balsamina (C)	Leaves	21	18	30
	Flowers	13	11	23
I. balsamina (T)	Leaves	32	23	58
	Flowers	20	18	38

(C-Control and T-Treatment)

The extraction yield is the measure of solvent efficiency to extract the specific component from the original material. Our results showed that maximum yield was obtained in methanolic extract of treated plant leaves (58%) followed by flower extract of the treated plant (38%). Methanol was shown to be an efficient solvent for the phytochemical extraction in comparison with than other solvents. The percentage yield was also different according to solvent used for extraction and method used for extraction.

Phytochemical analysis:
There was a statistically significant effect of Nickel (p=0.005) in comparison with solvent extract (p=0.061) and plant part (p=0.345) for the tested phytochemical constituents of *Impatiens balsamnia*. More numbers of phytochemical constituents were detected in Nickel treated (leaves [17] and flower extracts [15]) than in comparison with controls (leaves [6] and flower extracts [2]). The leaf and stem of *I. balsamnia* showed varying content of phytochemicals that were tested [57].

Table 2: Phytochemical constituents of different extracts of *I balsamina* leaves and Flower

Phytochemical test	Leaves						Flower					
	C			T			C			T		
Solvent	*H*	*Aq*	*M*	*H*	*Aq*	*M*	*H*	*Aq*	*M*	*H*	*Aq*	*M*
Alkaloid	-	+	+	-	+	+	-	-	+	-	-	+
Glycosides	+	-	-	+	-	+	-	-	-	+	-	+
Saponins	-	+	-	-	-	-	-	-	-	-	-	-
Sterols	-	-	-	-	+	+	-	-	-	-	-	+
Phenol	-	-	-	+	-	+	-	-	-	-	-	+
Flavonoids	+	-	-	+	-	+	+	-	-	-	+	+
Tannins	-	-	-	-	-	+	-	-	-	+	-	+
Quinones	-	-	-	+	+	+	-	-	-	+	-	+
Terpenoid	-	-	-	-	-	+	-	-	-	-	-	+
Anthocyanin	-	-	+	+	-	+	-	-	-	+	+	+

Key: + presence, - absence (H- Hexane, Aq.- Aqueous, M- Methanol, C- Control and T - Treatment)

In total, out of ten phytochemicals that were tested in the study in the leaves, nine (Alkaloid, Glycoside, Sterols, Phenol, Flavonoids, Tannin, Quinone, Terpenoid and Anthocyanin) were detected in methanolic extracts, five (Glycoside, Phenol, Flavonoids, Quinones and Anthocyanin) in hexane extracts and three in aqueous extracts of the Nickel treated leaves followed by only three (Alkaloids, Sterols and Quinones) types of phytochemicals were detected in aqueous extract. Similarly, in the flowers nine (Alkaloid, Glycoside, Sterols, Phenol, Flavonoids, Tannin, Quinone and Anthocyanin) were detected in methanolic extracts, four (Glycoside, Tannin, Quinones and Anthocyanin) in hexane extracts and two (Flavonoid, Anthocyanin) in aqueous extracts of the Nickel treated. Only one type of phytochemical was detected in the controls of methanolic and hexane extracts of flowers, including none of the tested phytochemicals in the aqueous extracts.

Majorly, alkaloids were detected in three of the various extracts of leaves including both control and heavy metal treated, followed by flavonoids and anthocyanins in three leaf extracts. Flavonoids and

glycosides were detected in methanolic extracts of Ni treated plants in comparison with its control, but interestingly, they were also detected in both the treatments of hexane extracts and undetected in aqueous extracts. Tumova & blazkova [58] has shown that, there was an increase of flavonoids in *Ononsis avrensis* in presence of Ni, Co and Cr. Anthocyanins and flavones of *Hibiscus sabdariffa* were increased under the Co and Ni stress [59]. *Gynura procumbens* when exposed to the Cadmium stress the total phenolic and flavonoid content was increased (60). Various heavy metals including Ni contaminated municipal waste increased the polyphenol content of *Mesembryanthemum edule* [61]. Sani Ahmad Jibri *et al*., [62] reported that high levels/concentration of Cadmium (Cd) and Ni stress increased the production of flavonoids, phenolics, and malondialdehyde. Saponins (antioxidant) were detected in the aqueous extract of leaf but not in the flower. The antioxidants were shown to vary in the plant parts (leaf, stem, flower and fruit) of various medicinal plant species (*Argemone mexicana, Datura metel, Calotropis procera, Thevetia peruviana,* and *Cannabis sativa*) [63]. Similarly, the methanolic and ethyl acetate extracts of *Callistemon citrinus* were shown to differ in the various bioactive components that were detected [64]. Alkaloids and glycosides were not detected in the whole plant ethanolic extracts of various *Impatiens* plant species including *I. balsamnia* [65]. But in our study, we were able to detect alkaloids and glycosides in the methanolic as well as hexane extracts. There was an effect of plant part and solvent on the detection of the various phytochemicals that were detected from the various plant parts tested.

Antibacterial activity of leaf and flower extracts

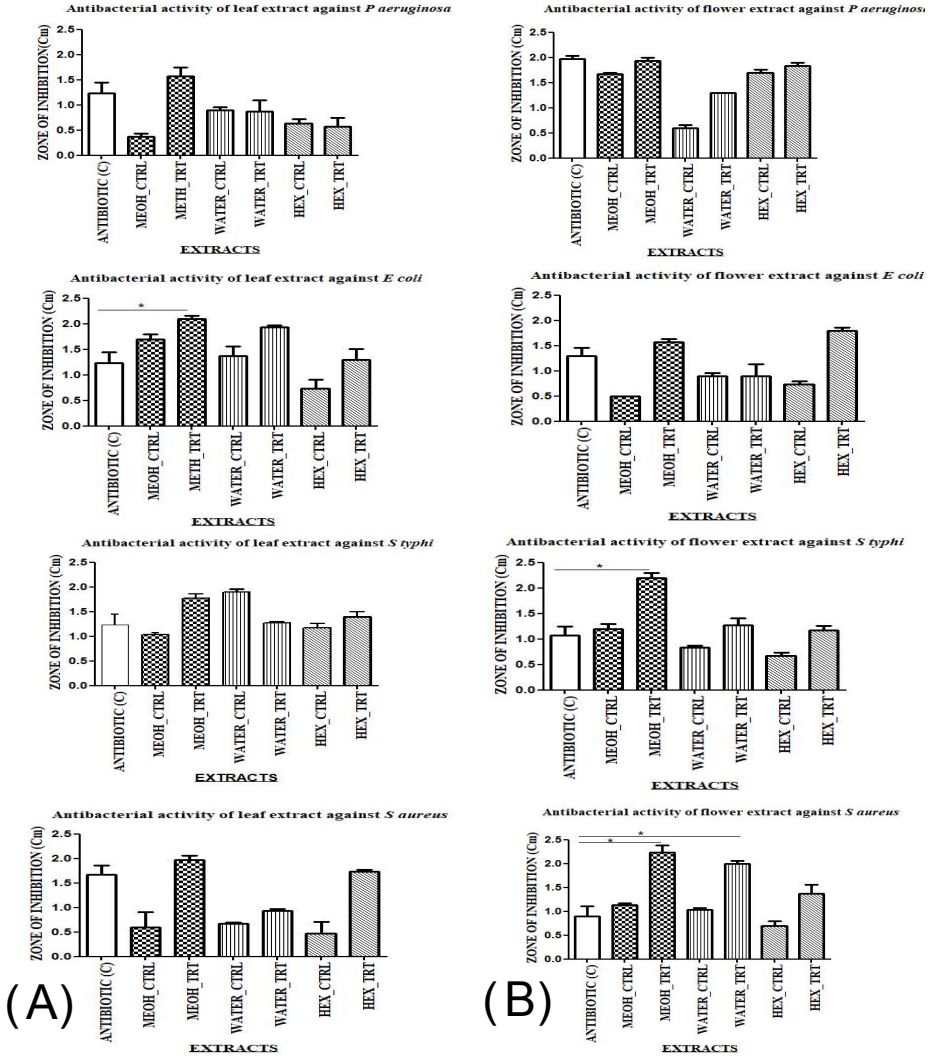

Figure 1. Inhibition potential of the *I. balsamnia* leaf and flower extracts. '*'represents the statistically significant difference ($p \leq 0.05$). Statistically significant difference in between antibiotic control and treatment were only represented here.

The zone of inhibition (ZOI) was found to be statistically significantly increased or more in most of the 'Ni' treated plant extracts when compared with antibiotic control. Several plants from *Impatiens* genus have been identified for the source of important metabolites with several showing antimicrobial activity, especially against Gram positive bacteria [66]. According to Meenu *et al*., [67] *I. balsamina* contains large amounts of phenols, flavonoids, proteins, carbohydrate, alkaloids, glycosides, iridoid glycosides, phenylethanoids, oligosaccharides, quinine, saponins, steroids, triterpenoids, sesquiterpenoids, and tannins, and also reported antimicrobial activity of its various extracts against various tested microorganisms. Antimicrobial activity of *I. balsamina* was reported due to greater quantities of alkaloid, flavonoid, tannins and phenol [68]. Henry *et al*., [69] showed that silver nanoparticle synthesized from the fresh leaves of *I. balsamina* leaves were more effective for antimicrobial activity than ciprofloxacin (control).

Overall, there was no statistically significant difference in between the plant parts (p=0.586) and organism tested (p=0.729), followed by a significant effect in solvent extract and 'Ni' treated (p=0.003). There was no statistically significant difference in the ZOI in between the flower and leaf irrespective of the extract type. But when measured within the plant parts, the organism and the 'Ni' treated showed a statistically significant effect (p=0.000) but not the extract. With respect to solvent extracts (overall) statistically, significant differences were observed in between methanolic extract with aqueous and hexane extracts (p≤0.03) but not in between Aqueous and Hexane. Based upon the organisms (overall), there was a statistically significant difference in ZOI in between *S. typhi* and *S. aureus* (p≤0.048). Considering the control and test samples at the whole (irrespective of other treatments), there was also a statistically significant difference (p≤0.000) in between all of them. The antibacterial property can also be attributed to certain plant metabolites such as naphthoquinones or hennotannic acid that were reported to be present in the leaves and flowers of *I. balsamnia* [70].

In most cases, the methanolic extract (irrespective of leaf or flower) 'Ni' treatment showed a statistically significant increase or increasing trend in the ZOI compared with its antibiotic treatment (p≤0.05). In a previous study, the ethanolic extracts of *I. balsamnia* leaves showed a higher amount of antimicrobial activity than the stem [57]. But in the present study, when compared with the leaf extracts, the flower extracts showed a higher statistically significant difference in between control and treatment against the tested organisms. Such difference can be attributed to the different solvent that were tested. The methanolic extracts of 'Ni' treated showed the presence of all the tested phytochemicals except the saponins and may be playing vital a role in inhibition. In *I. balsamina* presence of tannins were attributed to the wound healing properties and quinones also have the capacity to prevent bacteria to penetrate the wound [71]. The phenol and phenolic compounds have also been widely used in disinfection [72]. In antimicrobial activity tests, the leaf extracts *I. balsamnia* showed a higher amount of inhibition in comparison with stem against both gram-positive and gram-negative bacteria [57]. There was also an effect of solvent and organism on the zone of inhibition. For e.g., there was a statistically significant increase in the 'Ni' treated aqueous flower extracts that were able to inhibit *S. aureus* and *P. aeruginosa* but not with other organisms in comparison with its control extracts. The control extracts were found to be less inhibitory in comparison with the antibiotic control and the heavy metal treatment of plant extracts. By disc diffusion method solvent extracts of methanol *I. balsamnia*, acetone, petroleum ether, aqueous, acetone and hexane were tested upon on six human pathogens *Shigella boydii, Salmonella paratyphii, Proteus vulgaris, Staphylococcus aureus, Candida albicans* and *Cryptococcus neoformans*; of these, only *Shigella boydii, Candida albicans,* and *Cryptococcus neoformans* showed higher amount of inhibition in comparison with the others [73]. Of all the various plant parts (root/stem/leaf, seed, and pod) of *I. balsamnia* extracts, the pod extracts of acetone and ethyl acetate showed the higher amount of inhibition against multi drug resistant *Helicobacter pylori* in comparison with others. But in the present study the inhibition effects of flower 'Ni' treated was more inhibitory than leaf extract of 'Ni' treated plants. The hexane extracts of the *I. balsamnia* seed were shown to inhibit *S. aureus, Klebsiella pneumonia, Proteus vulgaris,* and *Serratia marcescens* [51]. In the present study also, there was a statistically significant increase in the zone of inhibition of heavy metal treated hexane extracts of leaves against *P. aeruginosa and S. aureus* when compared with its control.

Table 3. Minimal Inhibitory Concentration (µg/ml) of different extract of plant *I. balsamnia*

Extract (µg/ml)	Leaves				Flower			
	EC	*PA*	*SA*	*ST*	*EC*	*PA*	*SA*	*ST*
MC	1000	1000	1000	1000	1000	750	750	1000
MT	250	250	750	250	500	250	500	500
HC	750	1000	750	500	1000	1000	1000	750
HT	250	500	250	250	750	750	500	500
Aq. C	750	1000	750	750	1000	750	500	750
Aq. T	500	750	250	250	500	500	250	500

(*EC- Escherichia coli*, *PA- Pseudomonas aeruginosa*, *SA- Staphylococcus aureus*, *ST- Salmomella typhi*, MC-Methanol, control, MT-Methanol Treatment, HC-Hexane Control, HT-Hexane Treatment, Aq. C- Aqueous Control and Aq. T- Aqueous Treatment)

MIC results indicated that out of the four bacterial strains tested E coli was most sensitive, with the MIC values 250 µg/ml of leaves extract of the treated plants as compare to control plant and for flower extract is gives 500 µg/ml for treated plant. For control plant extract (leaves and flower) the MIC was at least more than double in most cases (750-1000 µg/ml) for all the bacterial strains tested as compared to the 'Ni' treated plant that showed 250-500 µg/ml of MIC.

Heavy metal analysis

According to Behera & Bhattacharya, (74) the plant-based medicines are said to be completely free from toxic metals and cross effect of allopathic drugs. WHO reported that medicinal plant products should check for the quality and quantity of the heavy metals (75). Deng *et al*., (76) has shown that the range of Nickel is varying ranging from 10mg/kg to >1000 mg/kg of the plant dry matter. Zoya *et al*., (77) has shown that Nickel is essential micro nutrient for plant and required in the amount of <0.5 mg/kg. In our study we found that the concentration of Nickel in the leaves of treated plants were 37 µg/gm and in the flower, it was 69 µg/gm i.e., below the permissible limit of Nickel. Faruk Karahan *et al*., (78) has shown that the concentration of Nickel that was found in the herb *Ferula communis* was 35.732 ± 0.47 mg/kg. In a study by Mahmood N *et al.,* (79), Nickel was detected below the permissible limits in Syzygium *aromaticum* flower buds (0.825 mg/kg) and in *Aegle marmelos* fruits (0.55 mg/kg).

CONCLUSION

The present study shows that there was a significant effect of Nickel on the phytochemicals of *Impatiens balsamnia.* Out of all the extracts methanolic extracts were effective and maximum number of phytochemicals were detected in comparison with other extracts. These Nickel treated plant extracts were shown to be effective in inhibiting the bacteria whose members are known to be pathogenic. Such plant extracts might show up some novel antimicrobials that might not be previously reported. But such studies need to be further validated using molecular and chemical methods.

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CONFLICT OF INTEREST

No conflict of interest is associated with this work.

REFERENCES

1. Janssens, S., Geuten, K., Yuan, M., Song, W., Yi, K., Philippe. & Smets, E. (2006). Phylogenetics of Impatiens and Hydrocera (Balsaminaceae) Using Chloroplast atpB-rbcL Spacer Sequences. Syst. Bot.,31:171-180.
2. Sinha, S.C. (1996).Medicinal plants of Manipur, Imphal. Manipur Association for Science and Society.,93:12.
3. Purkayastha, J., Nath, S.C. (2006).Biological activities of ethnomedical claims of some plant species of Assam. Indian J. Tradit. Knowl.,5:229-236.
4. Imam, M.Z., Nahar, N., Akter, S., & Rana, M.S. (2012).Antinociceptive activity of methanol extract of flowers of *Impatiens balsamina*. J. Ethnopharmacol.,*142*:804–810.
5. Chopra, R.N., Nayar, S.L. & Chopra, I.C. (1986).Council of Scientific and Industrial Research, New Delhi.
6. Hua, L., Peng, Z., Chia, L.S., Goh, N.K. & Tan, S.N. (2001).Separation of kaempferols in *Impatiens balsamina* flowers by capillary electrophoresis with electrochemical detection. J. Chromatogr A.,909(2):297–303.
7. Li, Q., Guo, Z., Wang, K., Zhang, X., Lou, Y. & Zhao, Y Q. (2015).Two new 1, 4-naphthoquinone derivatives from Impatiens balsamina L. flowers. Phytochem. Lett.,14:8–11.

8. Lei, J., Qian, S., Jiang, J. (2010).A new ursane caffeoyl ester from the seeds of Impatiens balsamina L. J. China Pharmceutical Univ.,41:118–119.
9. Koleva, I.I., Van Beek, T.A., Linssen, J.P.H. ,de Groot, A. & Evstatieva, L.N. (2002).Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Analysis.,13(1):8–17.
10. Yang, X., Summerhurst, D.K., Koval, S.F., Ficker, C., Smith, F.L. & Bernards, M.A. (2001).Isolation of an antimicrobial compound from Impatiens balsamina L. using bioassay-guided fractionation. Phytother. Res.,15:676–689.
11. Sakunphueak, A., Panichayupakaranant, P. (2012).Comparison of antimicrobial activities of naphthoquinones from Impatiens balsamina. Nat. Prod. Res.,26:1119–1124.
12. Kang, S.C., Moon, Y. (1992).Isolation and antimicrobial activity of a naphthoquinone from Impatiens balsamina. Kor. J. Pharmacogn.,23:240–247.
13. Kim, I.S., Yang, M.R., Lee, O.H. & Kang, S.N. (2011).Antioxidant Activities of Hot Water Extracts from Various Spices. Int. J. Mol. Sci.,12:4120–4131.
14. Lee, O.H., Lee, B.Y. (2010).Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. Bioresour. Technol.,101:3751–3754.
15. Manjunath, M., Lavanya, G, Sivajyothi, R. & Vijayasarathi, R.O. (2013).Activity-guided isolation and identification of anti-staphylococcal components from Senecio tenuifolius Burm. F. leaf extracts. Asian Pac. J. Trop. Biomed.,3(3):191-195.
16. Murthy, H.N., Lee, E.J. & Paek, K.Y. (2014).Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. Plant. Cell. Tiss. Org.,118:1–16.
17. Verpoorte, R., Contin, A. & Memelink J. (2002).Biotechnology for the production of plant secondary metabolites. Phytochem. Rev.,1:13–25.
18. Manjunatha, L., Kumar, V., Sannabommaji, T., Poornima, D V., Rajashekar, J. & Gajula, H. (2011).Influence of Nickel Treatment on Antioxidant Responses and Secondary Metabolite Production in Eryngium foetidium Linn. Ind. J. Pure App. Biosci.,7(5):314-326.
19. Xu,J., Yin, H.X. & Li, X. (2009).Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator Solanum nigrum L. Plant Cell Rep.,28:325– 333.
20. Denholm, J. (2010).Complementary medicine and heavy metal toxicity in Australia. Web. Med. Central.,1:1–6.
21. Tadiboyina, R., Ptsrk, P.R. (2016).Trace analysis of heavy metals in ground waters of Vijayawada industrial area.,1:3215-3229.
22. Kwaya, M.Y., Hamidu, H., Mohammed, A.I., Abdulmumini, Y.N., Adamu, I.H., Grema, H.M., Dauda, M., Halilu, F.B. & Kana, A.M. (2019).Heavy Metals Pollution Indices and Multivariate Statistical Evaluation of Groundwater Quality of Maru Town and Environs. J. Mater. Environ. Sci.,10(1):32-44.
23. Marschner, H. (1995).Mineral nutrition of higher plants, Academic Press, London. 889.
24. Khoshgoftarmanesh A.K., Bahmanziari, H. (2012).Stimulating and toxicity effects of nickel on growth, yield, and fruit quality of cucumber supplied with different nitrogen sources. J. Plant Nutr. Soil Sci.,175(3):474–481.
25. Da Silva, J.A.T., Naeem M. & Idrees M. (2012).Beneficial and toxic effects of nickel in relation to medicinal and aromatic plants. Med. Aromat. Plant Sci. Biotechnol.,6(1): 94–104.
26. Rodríguez, E.N., McLaughlin, M. & Pennock, D. (2018).Soil Pollution: a hidden reality. Rome, FAO. 142 pp.
27. Monali, P., Manoj, K. (2020).Assessment of metal contamination by using pollution indices in groundwater sources that used for drinking purpose in Olpad taluka, surat, India. I.J.A.R.I.I.T.,2454-132.
28. Sreekanth, T.V.M., Nagajyothi, P.C., Lee, K.D. & Prasad, T.N. (2013).Occurrence, physiological responses and toxicity of nickel in plants. Int. J. Environ. Sci. Technol.,10(5):1129–1140.
29. Shahzad, B., Tanveer, M., Rehman, A., Cheema, S.A., Fahad, S., Rehman, S. & Sharma, A. (2018).Nickel; whether toxic or essential for plants and environment – a review. Plant. Physiol. Biochem.,132:641–651.
30. Khan, W.U., Ahmad, S.R., Yasin, N.A., Ali, A., Ahmad, A. & Akram, W. (2017).Application of Bacillus megaterium MCR-8 improved phytoextraction and stress alleviation of nickel in Vinca rosea. Int. J. Phytoremediation.,19(9):813–824.
31. Manjunatha, L., Vadlapudi, K, Torankumar., Poornima, D.V., Rajashekar, J. & Hari, G. (2019).Influence of Nickel Treatment on Antioxidant Responses and Secondary Metabolite Production in Eryngium foetidium Linn. Ind. J. PureApp. Biosci.,7(5):314-326.
32. Zahra, S.N., Abolfazl, A., Parviz, N. (2021).Effect of foliar application of nickel on physiological and phytochemical characteristics of pot marigold (Calendula officinalis). J. Agri. Food Res.,3:100-108.
33. Soliman, M., Alhaithloul, H.A., Hakeem, K.R., Alharbi, B.M., El, E.M. & Elkelish, A. (2019).Exogenous Nitric Oxide Mitigates Nickel-Induced Oxidative Damage in Eggplant by Upregulating Antioxidants, Osmolyte Metabolism, and Glyoxalase Systems. **Plants**.,8(12):562.
34. Wang, X.F., Zhou, Q.X. (2005).Ecotoxicological effects of cadmium on three ornamental plants. Chemosphere.,60:16–21.
35. Weitao, L., Jiani, W., Jiapan, L., Xue, Z., Aurang, Z., Qixing, Z. & Yuebing, Sun. (2020).Potential use of Impatiens balsamina L. for bioremediation of lead and polychlorinated biphenyl contaminated soils. Land Degrad Dev.,1–12.
36. Gopal, R., Neelam, C., Tapan, A. (2014).Nickel as a Pollutant and its Management. Int. Res. J. Environ. Sci.,3(10): 94-98.

37. Amin, G., Nallanchakravarthula, S. (2018).Plant growth promotion and inhibitory potential of fluorescence (under UV light) exhibiting root endophytic bacteria isolated from Abelmoschus esculentus (Okra) cultivars. European J. Biotechnol. Biosci.,6:18-26.
38. Oluwaseun, R., Alara, N.H., Abdurahman, C.I. & Ukaegbu. (2018).Soxhlet extraction of phenolic compounds from Vernonia cinerea leaves and its antioxidant activity. Journal of Applied Research on Medicinal and Aromatic Plants.,11:12-17.
39. Osman, A., Ragaa, S., Mohmmed, A. & Saad, M.H.A. (2019).The Effect of Extraction method and Solvents on yield and Antioxidant Activity of Certain Sudanese Medicinal Plant Extracts. J. Phyto.,8(5): 248-252.
40. Ameyam, Y., Duker, E.G. (2009).The alkaloid contents of the ethno-plant organs of three antimalarial medicinal plant Species in the eastern region of Ghana. Int. J. Chem. Sci.,**7:**48-58.
41. Rajan, S., Thirunalasundari, T. & Jeeva, S. (2011).Antienteric bacterial activity and phytochemical analysis of the seed kernel extract of Mangifera indica Lin. against Shigella dysenteriae (Shiga, corrig.) Castellani and Chalmers. Asian Pac. J. Trop. Med.,4(4): 294–300.
42. Harborne, J.B. (1973).Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd. Ltd. London. pp.49.
43. Meriga, B., Mopuri, R., Krishna, T.M. (2012).Insecticidal,antimicrobial and antioxidant activities of bulb extracts of Allium sativum. Asian Pac. J. Trop. Med.,5(5): 391–395.
44. Mercy, G.A., Light, F.W., Gospel, A. (2017).Qualitative and Quantitative Phytochemical Screening of Some Plants Used in Ethnomedicine in the Niger Delta Region of Nigeria. J. Food Nutr. Sci.,5(5):198-205.
45. Okerulu, I.O., Onyema, C.T., Onwukeme, V.I. & Ezeh C.M. (2017).Assessment of phytochemicals, proximate and elemental composition of Pterocarpus soyauxii (Oha) Leaves. Am. J. Analyt. Chem.,80:406-415.
46. Oyewole, O., Akingbala, P.F. (2011).Phytochemical analysis and hypolipidemic properties of jatropha tanjorensis leaf extract. European J. Med. Plants.,1(4):180–185.
47. Parekh, J., Chanda, S.V. (2007).In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk. J. Biol.,31:8-53.
48. Middha, S.K., Usha, T. & Pande, V. (2013).HPLC evaluation of phenolic profile, nutritive content and antioxidant capacity of extracts obtained from Punica granatum fruit peel. Advances in Pharmacological Sciences. Volume 2013,Article ID 296236.
49. Jamil, M., Mirza, B., Yasmeen, A., Khan, M.A. (2012).Pharmacological activities of selected plant species and their phytochemical analysis. J. Med. Plants Res.,6:5013-5022.
50. Mufti, F.D., Hnif, U. Bangash, A., Khan, N., Hussain, S. & Farhat, Ullah. (2012).Antimicrobial activities of Aerva javanica and Paeonia emodi plants. Pak. J. Pharm. Sci., 5(3): 565–569.
51. Manikandan, A., Rajendran, R., Abirami, M. & Kongarasi, K. (2016).Antimicrobial activity and phytochemical analysis of Impatiens balsamina seed (kaci-tumpi) collected from coimbatore district, tamilnadu, India. Int. J. Pharm. Sci. Res.,7(12):5039-5043.
52. Manjunath, M., Sharma, P.V.G.K. & Reddy, O.V.S. (2008).In Vitro Evaluation Of Antibacterial Activity Of Actiniopteris Radiata (Sw.) Link. J. Pharm. Chem.,2(2):112-117.
53. Timm, M., Saaby, L., Moesby, L. & Hansen, E.W. (2013).Considerations regarding use of solvents in in vitro cell based assays. Cytotechnology.,65:887-894.
54. NCCLS (National Committee of Clinical Laboratory Standards). (2002).National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts Proposed standard,M27-A2.
55. Clinical and Laboratory Standards Institute (CLSI). (2015).Performance standards for Antimicrobial Disk Susceptibility Tests; 364 Approved Standard – Twelfth Edition. Wayne, PA: Clinical and Laboratory Standards Institute.
56. Sanjay, G., Tiku, A.K., Apurva, K., Sahil, G., Gurjinder, S. & Razdan, V.K. (2013).Antioxidant and Antimicrobial Properties of the Essential Oil and Extracts of Zanthoxylum alatum Grown in North-Western Himalaya. Hindawi Publishing Corporation. The Scientific World Journal Volume, http://dx.doi.org/10.1155/2013/790580.
57. Kang, S.N., Goo, Y.M., Yang, M.R., Ibrahim, R.I.H., Cho, J.H., Kim, I.S. & Lee, O.H. (2013).Antioxidant and antimicrobial activities of ethanol extract from the stem and leaf of Impatiens balsamnia L. (Balsamniaceae) at different harvest times: Molecules.,18:6356-6365.
58. TumovaBlazkova, R. (2002).Effect on the formation of flavonoids in the culture of Ononis arvensis L. in vitro by the action of CrCl3. Ceska. Slov. Farm.,51:44-46.
59. Eman, A., Gad, N., & Badran, N.M. (2007).Effect of cobalt and nickel on plant growth, yield and flavonoids content of Hibiscus sabdariffa L. Aus. J. Basic. Appl. Sci.,1:73–78.
60. Mohd, H.I., Yap, C.K., Nurul, A. & Mohd, Z. (2017).Effect of Cadmium and Copper Exposure on Growth, Secondary Metabolites and Antioxidant Activity in the Medicinal Plant Sambung Nyawa (*Gynura procumbens* (Lour.) Merr). Molecules.,22:16-23.
61. Lakhdar, A., Falleh, H., Ouni, Y., Ksouri, R. & Abdelly, C. (2011).Municipal solid waste compost application improves productivity, polyphenol content, and antioxidant capacity of Mesembryanthemum edule. J. Hazard. Mater.,191:373–379.
62. Sani, A.J., Siti, A.H., Che, F.I. & Puteri, E.M.W. (2017).Cadmium Toxicity Affects Phytochemicals and Nutrient Elements Composition of Lettuce (Lactuca sativa L.): Hindawi Advances in Agriculture Volume., Article ID 1236830, 7 pages https://doi.org/10.1155/2017/1236830.

63. Srivastava, N., Chauhan, A.S., & Sharma, B. (2012).Isolation and Characterization of Some Phytochemicals from Indian Traditional Plants: Biotechnology Research International.https://doi: 10.1155/2012/549850.
64. Arayetam, R., Zacchaeus, S.O., Oluranti, O.O. & Ayodele, L. (2019).Phytochemical Constituents, Antioxidant, Cytotoxicity, Antimicrobial, Antitrypanosomal, and Antimalarial Potentials of the Crude Extracts of Callistemon citrinus: Evid. Based Complementary Altern., Med. https://doi.org/10.1155/2019/5410923.
65. Delgado, R.F.V., Hidalgo, O., Loria, G.A., Weng, H.N.T. (2017).*In vitro* antioxidant and antimicrobial activities of ethanolic extracts from whole plants of three *Impatiens* species (balsaminaceae). Ancient. Sci. Life;37:16-23.
66. Su, B.L., Zeng, R., Chen, J.Y., Chen, C.Y., Guo, J.H. & Huang, C.G. (2012).Antioxidant and antimicrobial properties of various solvent extracts from Impatiens balsamina L. stems. J. Food. Sci.,77:614-619.
67. Meenu, B., Neeraja, E.D., Greeshma, R. & Alexeyena, V. (2015).*Impatiens balsamina*: an overview. J. Chem. Pharm. Res.,7(9):16–21.
68. Osunton, O.T., Ajayi, A. (2014).Antimicrobial, Phytochemical and Proximate analysis four Nigerian medicinal plantson some clinical microorganisms. Current research in microbioloy and biotechnology.,2 (5): 457-461.
69. Henry, F., Aritonang, Harry, K., Audy, D. & Wuntu. (2019).Synthesis of Silver Nanoparticles Using Aqueous Extract of Medicinal Plants' (Impatiens balsamina and Lantana camara) Fresh Leaves and Analysis of Antimicrobial Activity. Int. J. Microbiol.,2019, Article ID 8642303, 8.
70. Sakunphueak, A., Panichayupakaranant, P. (2010). Simultaneous determination of three naphthoquinones in the leaves of *Impatiens balsamina* L. by reversed-phase high-performance liquid chromatography. Phytochem. Anal.,21(5):444–50.
71. Hariyanto, I.H., Kusharyanti, I. & Iwo, M.I. (2016).Antiarthritic activity of pacar air (Impatiens balsamina Linn.) herb extract in animal model of rheumatoid arthritis-autoimmune disease. Int. J. Pharm. Tech. Res.,9(3):131-137.
72. Oku, H., Ishiguro, K. (2011).Antipruritic and antidermatitic effect of extract and compounds of Impatiens balsamina L. in atopic dermatitis model NC mice. Phytother Res.,15(6):506-510.
73. Ahmed, S., Koperuncholan, M. (2012).Antibacterial activities of various solvent extracts from *Impatiens Balsamina*. Int. J. Pharm. Bio Sci.,3(2):401-406.
74. Behera, B., Bhattacharya, S. (2016).The importance of assessing heavy metals in medicinal herbs: a quantitative study. Humanitas Medicine.,6(1):1-4.
75. Singh, K.P., Bhattacharya, S. & Sharma, P. (2014).Assessment of heavy metal contents of some Indian medicinal plants. Am. Eurasian J. Agric. Environ. Sci., 14(10):1125- 1129.
76. Deng, H., Ye, Z.H. & Wong, M.H. (2004).Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. Environ. Pollut.,132(1):29–40.
77. Zoya, G., Hira, I., Muhammad, F., Bhatti., Nasar, U.M., Iti, S., Alvina, G,. Kazi. & Parvaiz. (2016).Phytoextraction In: The use of plants to remove heavy metals from soil. Elsevier Inc.,385–409.
78. Faruk, K. & Ibrahim, I.O., Ibrahim, A.S., Ibrahim, E.Y., Asli, H.O. & Ahmet. I. (2019).Heavy Metal Levels and Mineral Nutrient Status in Different Parts of Various Medicinal Plants Collected from Eastern Mediterranean Region of Turkey. Biol. Trace Elem. Res., https://doi.org/10.1007/s12011-019-01974-2.
79. Mahmood, N., Nazir, R., Khan, M., Khaliq, A., Adnan, M., Ullah, M. & Yang, H. (2019).Antibacterial Activities, Phytochemical Screening and Metal Analysis of Medicinal Plants: Traditional Recipes Used against Diarrhoea. Antibiotics.,8(4):194.

Supplementary table 1. Statistically significant difference in the leaf extracts of IB

S.No	Leaf							
1	***E.coli (EC)***	LEC_AB_C	LEC_WC	LEC_WT	LEC_MC	LEC_MT	LEC_HC	LEC_HT
	LEC_AB_C	NS	NS	NS	NS	*	NS	NS
	LEC_WC			NS	NS	NS	NS	NS
	LEC_WT				NS	NS	*	NS
	LEC_MC					NS	*	NS
	LEC_MT						*	*
	LEC_HC							NS
2	***S typhi* (ST)**	LST_AB_C	LST_WC	LST_WT	LST_MC	LST_MT	LST_HC	LST_HT
	LST_AB_C		NS	NS	NS	NS	NS	NS
	LST_WC			NS	*	NS	NS	NS
	LST_WT				NS	NS	NS	NS
	LST_MC					*	NS	NS
	LST_MT						*	NS
	LST_HC							NS
3	***S aureus* (SA)**	LSA_AB_C	LSA_WC	LSA_WT	LSA_MC	LSA_MT	LSA_HC	LSA_HT
	LSA_AB_C		*	NS	*	NS	NS	NS
	LSA_WC			NS	NS	*	NS	*
	LSA_WT				NS	*	NS	*
	LSA_MC					*	NS	*
	LSA_MT						NS	NS
	LSA_HC							*
4	**P aeruginosa (P)**	LP_AB_C	LP_WC	LP_WT	LP_MC	LP_MT	LP_HC	LP_HT
	LP_AB_C		NS	NS	NS	NS	NS	NS
	LP_WC			NS	NS	NS	NS	NS
	LP_WT				NS	NS	NS	NS
	LP_MC					*	NS	*
	LP_MT						NS	NS
	LP_HC							*

'*' significant difference ($p \leq 0.05$), 'NS' non-significant difference

Supplementary table 2. Statistically significant difference in the flower extracts of IB

S.No	Flower							
1	***E.coli* (EC)**	FEC_AB_C	FEC_WC	FEC_WT	FEC_MC	FEC_MT	FEC_HC	FEC_HT
	FEC_AB_C		NS	NS	*	NS	NS	NS
	FEC_WC			NS	NS	NS	NS	*
	FEC_WT				NS	NS	NS	*
	FEC_MC					*	NS	*
	FEC_MT						*	NS
	FEC_HC							*
2	***S typhi* (ST)**	FST_AB_C	FST_WC	FST_WT	FST_MC	FST_MT	FST_HC	FST_HT
	FST_AB_C		NS	NS	NS	*	NS	NS
	FST_WC			NS	NS	*	NS	NS
	FST_WT				NS	NS	NS	NS
	FST_MC					*	NS	NS
	FST_MT						*	*
	FST_HC							NS
3	***S aureus* (SA)**	FSA_AB_C	FSA_WC	FSA_WT	FSA_MC	FSA_MT	FSA_HC	FSA_HT
	FSA_AB_C		NS	*	NS	*	NS	NS
	FSA_WC			*	NS	*	NS	NS
	FSA_WT				NS	NS	*	NS
	FSA_MC					*	NS	NS
	FSA_MT						*	NS
	FSA_HC							NS
4	*P aeruginosa* (P)	FP_AB_C	FP_WC	FP_WT	FP_MC	FP_MT	FP_HC	FP_HT
	FP_AB_C		*	NS	NS	NS	NS	NS
	FP_WC			NS	*	*	*	*
	FP_WT				NS	NS	NS	NS
	FP_MC					NS	NS	NS
	FP_MT						NS	NS
	FP_HC							NS

'*' significant difference ($p \leq 0.05$), 'NS' non-significant difference