ORIGINAL ARTICLE

Stamford Journal of Microbiology, December 2014. Vol. 4, Issue 1, p. 19-23
ISSN: 2074-5346

Estimation of microbiological propagation and antimicrobial traits of the frequently accessible flowers

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Received 30 May 2014/Accepted 28 July 2014

Present study portrayed a complete microbiological profile of commonly available flowers including *Rosa kordesii, Gladiolus hybrid, Acmella oleracea, Nyctanthes arbortristis* and *Pseudomussaenda flava* which were randomly collected from Dhaka city, Bangladesh. The microbial contamination was quantified up to 10^8 cfu/g. Exploration of specific pathogenic bacteria was estimated within the range of 10^3 to 10^8 cfu/g of which *Pseudomonas* spp. was found in *G. hybrid, A. oleracea* and *P. flava* (~10^6 cfu/g), whereas *Escherichia coli* and Staphylococcal contamination was evident in almost all samples up to 10^8 cfu/g. The *in vitro* antimicrobial activities of the flower extracts were notable against most of the test bacteria. The ethanolic extracts of *R. kordesii* showed anti-bacterial activity against most of the bacteria except *E. coli* and *Salmonella* spp. *G. hybrid* extracts showed activity against *Klebsiella* spp. and *Bacillus* spp., *A. oleracea* against *E. coli*, *Pseudomonas* spp., *Bacillus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Salmonella* spp., *P. flava* against *Pseudomonas* spp. and *Bacillus* spp., and *N. arbortristis* against *Bacillus* spp. The methanol extracts of *G. hybrid* possessed activity against *E. coli*, *Listeria* spp. and *Pseudomonas* spp., *N. arbortristis* extracts against *E. coli*, *Vibrio* spp., *Bacillus* spp., *Klebsiella* spp. and *Staphylococcus* spp., *P. flava* extracts against *E. coli*.

Key words: Microbiological analysis; Flowers; Antimicrobial activity

Plants have the ability to synthesize a wide range of chemical components with numerous beneficial effects to treat human disease complications (1, 2). Apart from antibiotic therapy which is currently known to face the shortfall of drug- resistant microorganisms, use of herbal medicines gained its acceptance in recent days (3-11). Several studies have been reported to possess the antioxidant activity of plants with wound-healing effects as well as to prevent the formation and oppose the action of toxic radicals (8, 12-14). In global prospective, among the herbal products, uses of flower extracts for disease medication is not unlikely (11, 15).

Some common flowers like *Hibiscus rosasinensis* and *Ixora cocconea* have been reported to exhibit the antimicrobial activity against common pathogenic microorganisms (14, 16-20). *Ipomoea digitata* and *Allmanda cathartic* have long been used for the mitigation indigestion, tuberculosis, tumors, malaria and liver complications (21-23). However, along with their beneficial effects, microbiological contamination of flowers leading to disease commencement is a pitfall (24-26). Based on these facts, current investigation endeavored (i) to the simulation study of bacterial and fungal access to *Rosa kordesii*, *Gladiolus hybrid*, *Acmella oleracea*, *Nyctanthes arbortristis* and *Pseudomussaenda flava*, and (ii) to unravel the in vitro antimicrobial activity of those flower extracts.

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MATERIALS AND METHODS

Study area, sampling, sample processing and microbiological analysis. Five categories of flowers including *R. kordesii*, *G. hybrid*, *A. oleracea*, *N. arbortristis* and *P. flava*, were randomly collected during February 2013 to April 2013 following standard protocol (27). Samples were quickly transported to the laboratory, and prior to microbiological assay, 10 g of each sample was mixed with 90 ml of buffer peptone water (pH 7.2 ± 0.2) in 9:1 ratio and serially diluted up to 10^{-5}.

From the dilution 10^{-4} each of the samples 0.1 ml was introduced on to the nutrient agar and Sabouraud dextrose agar for the isolation of total viable bacteria and fungi, respectively. Subsequently, MacConkey agar, Membrane Fecal Coliform agar (M-FC), Manitol Salt agar, Cetrimide agar and Actinomycetes agar were used as selective media for quantification of coliforms, fecal coliforms, *Staphylococcus* spp. *Pseudomonas* spp. and Actinomycetes, consecutively (28-32). All the inoculated plates were incubated at 37 °C for 24 hours except SDA plates, which were incubated at 25 °C for 48 hours.

Solvent extraction. The dried parts (petals, steam and sepal) of each flower were first ground, and the fine powders were added to 120 ml of ethanol and methanol in Durham's bottle which were kept in shaking water bath at 130 rpm for 24 h. at 20 °C. Extracts were then concentrated in a rotary evaporator under reduced pressure. The dried residual extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to a final concentration of 10mg/ml (33-35). Samples were stored overnight at -20 °C until use.

Assay of antimicrobial activity. The Mueller-Hinton agar (MHA) plates were prepared followed by modified agar well diffusion method (31, 32, 36, 37). Lawns of bacterial suspensions including *Escherichia coli*, *Pseudomonas* spp., *Listeria* spp., *Vibrio* spp., *Klebsiella* spp., *Staphylococcus aureus*, *Bacillus* spp. (turbidity compared with the McFarland standard) were prepared, wells (8 mm^3) were made, and 100µl of the crude extract, ethanol- and methanol extracts at a concentration of ~11.1mg/ml each were introduced into the wells. Absolute ethanol, methanol and dimethyl sulfoxide (10%) were used as negative controls while the antibiotic discs of gentamicin (10 µl) were used as positive control. Plates were incubated at 37 °C for 12-18 hours and examined for formation of the zone of inhibitions (mm).

RESULTS AND DISCUSSION

Previously our group unveiled the anti-bacterial traits of *H. rosasinensis*, *I. cocconea*, *I. digitata* and *A. cathartic* extracts (unpublished) which is in consistent to several other studies (18-20). Since these flowers are widely known to combat against a range of disease complications, we further turned our attention to extend such investigation on other flowers easily available in Bangladesh. One important aspect is that in the view of the study of inhabitant microflora in flowers and herbs, not so much studies have been reported so far. Our study showed that almost all samples exhibited huge gathering of spoiling bacterial and fungal flora (Table 1). *G. hybrid*, *A. oleracea* and *P. flava* were found to be contaminated with *Pseudomonas* spp. (~10^6 cfu/g). *E. coli* and Staphylococcal contamination was extremely significant up to 10^7 and 10^8 cfu/g, respectively in all the tested samples. No growth of actinomycetes and *Klebsiella* spp. was observed. To our knowledge, such a quantification of flower accessing microorganisms is for the first time in Bangladesh.

Anti-bacterial activity of flower extracts. Several studies revealed flower extracts to be inhibitory of growth of certain pathogens including *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *S. pneumoniae*, and *Enterobacter aerogens* (38, 39). According to our study, the aqueous extract of *G. hybrid* and *N. arbortristis* were found to exhibit the bactericidal effects against *Salmonella* spp. (13mm) and *Staphylococcus* spp. (12mm) respectively, whereas extracts of *R. kordesii*, *A. oleracea*, *P. flava* showed no antimicrobial activity. The crude fraction and the residual extracts also showed no activity.

The ethanolic extracts of *R. kordesii* was found to form zone of inhibition against both Gram negative and Gram positive bacteria. However, no activity was scored against *E. coli* and *Salmonella* spp. (Table 2). In case of *G. hybrid* ethanol extract, the maximum activity was found on *Klebsiella* spp. and *Bacillus* spp. whereas no antibacterial activity was coined against others (Table 3).

The extracts of *A. oleracea* was found to be effective against *E. coli*, *Pseudomonas* spp., *Bacillus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Salmonella* spp. while *Vibrio* spp. and *Listeria* spp. were found to be resistant (Table 4). *N. arbortristis* was found to be active only against *Bacillus* spp. (Table 5) whereas the ethanolic extract of *P. flava* was active against *Pseudomonas* spp. and *Bacillus* spp. (Table 6).

Likewise, the methanol extracts of *G. hybrid* showed the anti-bacterial activity with the inhibition zones of 14mm, 13 mm and 10 mm against *E. coli*, *Listeria* spp. and *Pseudomonas* spp. consecutively (Table 3). The extracts of *N. arbortristis* were found to inhibit the growth of *E. coli*, *Vibrio* spp., *Bacillus* spp., *Klebsiella* spp. and *Staphylococcus* spp. (Table 5). *P. flava* extracts were found active only against *E. coli* (Table 6) while the extracts of *R. kordesii* and *A. oleracea* were inactive

TABLE 1. Microbiological profile of flower samples

Samples	**TVB (cfu/g)**	**Fungi (cfu/g)**	***E. coli* (cfu/g)**	***Staphylococcus* spp. (cfu/g)**	***Pseudomonas* spp. (cfu/g)**
Rosa kordesii (n=5)	3.2×10^8	4.4×10^8	2.2×10^6	2.6×10^8	0
Gladiolus hybrids (n=5)	6.5×10^8	3.5×10^8	5.0×10^5	4.4×10^8	2.2×10^6
Acmella oleracea (n=5)	3.4×10^8	3.6×10^8	3.9×10^4	3.7×10^8	4.1×10^3
Nyctanthes arbortristis (n=5)	5.0×10^8	4.8×10^8	4.4×10^7	2.0×10^8	0
Pseudomussaenda flava (n=5)	4.8×10^8	3.2×10^8	1.0×10^6	2.2×10^8	4.2×10^5

TVB = Total viable bacteria

The average microbial load has been shown in the table. Fecal coliform and *Klebsiella* spp. and actinomycetes were completely absent in all samples.

The experiment has been done in triplicate and the result was reproducible.

TABLE 2. Antimicrobial activity of *Rosa kordesii* extracts

Test bacteria	**Zone of Inhibition in diameter (mm)**						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10µg)
E. coli	0	0	8mm	0	0	0	16.80mm
Pseudomonas spp.	0	0	0	21mm	0	0	28.44mm
Vibrio spp.	0	0	0	14mm	0	0	18.01mm
Bacillus spp.	0	0	7mm	11mm	0	0	22mm
Klebsiella spp.	0	0	0	12mm	0	0	18.83 mm
Staphylococcus spp.	0	0	0	18mm	0	0	22mm
Listeria spp.	0	0	0	10mm	0	0	23.00mm
Salmonella spp.	0	0	0	0	0	0	29.22mm

TABLE 3. Antimicrobial activity of *Gladiolus hybrid* extracts

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10µg)
E. coli	0	0	8mm	11mm	0	14mm	16.80mm
Pseudomonas spp.	0	0	0	0	0	10mm	28.44mm
Vibrio spp.	0	0	0	0	0	0	18.01mm
Bacillus spp.	0	0	7mm	10mm	0	0	22mm
Klebsiella spp.	0	0	0	12mm	0	0	18.83 mm
Staphylococcus spp.	0	0	0	0	0	0	22mm
Listeria spp.	0	0	0	0	0	13mm	23.00mm
Salmonella spp.	0	0	0	0	0	0	29.22mm

TABLE 4. Antimicrobial activity of *Acmella oleracea* extracts

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10µg)
E. coli	0	0	8mm	11mm	0	0	16.80mm
Pseudomonas spp.	0	0	0	12mm	0	0	28.44mm
Vibrio spp.	0	0	0	0	0	0	18.01mm
Bacillus spp.	0	0	7mm	11mm	0	0	22mm
Klebsiella spp.	0	0	0	11mm	0	0	18.83 mm
Staphylococcus spp.	0	0	0	11mm	0	0	22mm
Listeria spp.	0	0	0	0	0	0	23.00mm
Salmonella spp.	0	0	0	15mm	0	0	29.22mm

TABLE 5. Antimicrobial activity of *Nyctanthes arbortristis* extracts

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10µg)
E. coli	0	0	11mm	0	0	12mm	16.80mm
Pseudomonas spp.	0	0	0	0	0	0	28.44mm
Vibrio spp.	0	0	0	0	0	11mm	18.01mm
Bacillus spp.	0	0	11mm	11mm	0	13mm	22mm
Klebsiella spp.	0	0	0	0	0	12mm	18.83 mm
Staphylococcus spp.	0	0	0	0	0	11mm	22mm
Listeria spp.	0	0	0	0	0	0	23.00mm
Salmonella spp.	0	0	0	0	0	0	29.22mm

TABLE 6. Antimicrobial activity of *Pseudomussaenda flava* extracts

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10µg)
E. coli	0	0	8mm	0	0	14mm	15mm
Pseudomonas spp.	0	0	0	14mm	0	0	14mm
Vibrio spp.	0	0	0	0	0	0	18mm
Bacillus spp.	0	0	7mm	11mm	0	0	22mm
Klebsiella spp.	0	0	0	0	0	0	18.83 mm
Staphylococcus spp.	0	0	0	0	0	0	22mm
Listeria spp.	0	0	0	0	0	0	15mm
Salmonella spp.	0	0	0	0	0	0	16mm

against all the test bacteria (Table 2 and Table 4).

From our findings, it is evident that the *in vitro* antimicrobial activities of the flower extracts, especially after ethanol treatment, were prominent against microorganisms. Also, interestingly the ethanol extract of all the samples exhibited antimicrobial activity against *Bacillus* spp. Presence of antimicrobial activity in the solvent extracts of the flower samples tested could possess the potential of being source of commercially dispensable herbal antimicrobial agents against different topical and enteric diseases (39-41).

CONCLUSION

A large number of studies have been carried out so far to detect the microbiological contamination level in flowers and others herbs; nevertheless, the sustainable capacity of such contamination is still now difficult to understand. Our study revealed a complete profile of microbial contamination in flower and measured the anti-bacterial activity of their ethanol and methanol extracts. The findings may largely assist to design a model for the development of new herbal medicines thereby augmenting the public health safety.

ACKNOWLEDGEMENTS

Authors are thankful to Stamford University Bangladesh for providing with financial aid and logistic supports.

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