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In vitro assessment of *Tabernaemontana crispa* on bacterial isolates from rural ducks in South Andaman

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Abstract

The present study was aimed to determine the antimicrobial activity of an endemic plant species used in folkloric medicine by the inhabitants of Andaman Islands, India. The methanolic extracts prepared from the leaves of, *Tabernaemontana crispa*, were assessed for antibacterial activity against clinically isolated human pathogenic bacterial activity. The Methonolic extracts showed more inhibition towards Gram negative bacteria. *In-vitro work was designed to determine antimicrobial effect of Tukrotong (Tabernaemontana crispa,) on duck feacal isolates from various place of South Andaman. Antibiogram in terms of zone of inhibition (ZI) of Methonolic extract of herbal extracts at concentrations (µg) against salmonella and E.coli isolates were performed. Extracts of Tukrotong methonlic (20 & 30 µg) exhibited significantly highest antibacterial activity (16.9 mm) against E.coli isolates and against the Salmonella isolates it was (16.6 mm). with The anti-bacterial properties of Methonolic extract of this medicinal plants have revealed that they could be further studied in poultry*

Keywords: endemic plants, antibacterial activity, herbal extracts, antibiogram, rural poultry.

Introduction

Ethno medicine is a study or comparison of the traditional medicine practiced by various ethnic groups, and especially by indigenous peoples. The word ethno medicine is sometimes used as a synonym for traditional medicine (Wikipedia). Plants have been used as source of medicine throughout the world for many years ago and still continue to occupy an important place in traditional as well as modern systems of medicine.

Traditional knowledge of medicinal plants and their use by the indigenous healers and drug development in the present are not only useful for conservation of cultural tradition and biodiversity but also for community health care and drug development in the local people. The indigenous knowledge on medicinal plants appears when humans started and learned how to use the traditional knowledge on medicinal plants.

Interestingly, the traditional system of medicine using plants as a source have acquired greater impetus in the last three decades as excessive use of synthetic drugs and antibiotics have been found to cause number of side effects. Therefore, the investigation on the medicinal plants is increasing progressively all over the world and more stress is being placed on herbal drugs. One of the commonly found plants that are used by tribal farming community is *Tabernaemontana crispa (*Tokuratong, local name) among Nicobari tribes. Hence, the invitro study was carried out to assess the antimicrobial activity of *Tabernaemontana crispa* for future use to develop herbal health care for rural poultry and ducks.

Materials and methods

A medicinal plant survey has been conducted in the selected localities e.g. Tamaloo, Perk (Car-Nicobar), and Harmander Bay (Little Andaman) and medicinal plants were collected. The local names and their medicinal uses were recorded with the help of some traditional herbal collectors, local vaidyas (N.T.-Kach roy chon) and Botanical Survey of India, Andaman (Table 1). Duck feacal samples were collected from two different parts of S-Andaman. i.e, Ferrargunj and Namunagar and their antibiotic sensitivity was tested. Antibacterial activity of the plant was screened against the isolates

Table 1: Plant used as Traditional Medicine by the Nicobari Tribes of Andaman & Nicobar Islands

Local name of Medicinal Plants	Latin name	Family	Part used	Disease Indications in common and Nicobari Terms	Mode of preparation/ Uses	Dose & Duration
Tokurotong (Koraiya)	*Tabernaemontana crispa Roxb.*	Apocynaceae	Leaf	Injury (Faar dare)	Prepared paste (Kalk) and applied externally	Once a day for 5-7 days

Extraction of medicinal plants

Methanolic extracts of herbal plants were prepared as per the method described by S´anchez*et al*. (2010). Plants Tokurotong (*Tabernaemontana crispa) were* collected from Nicobar and Hutbay Island and processed for methanol extraction. Collected samples were washed with tap water and powdered after drying under shadow. Samples were then soaked with periodical manual shaking in methanol at the ratio of 1:10 dilution for 3 days. After the period, it was filtered and kept at 40 – 50° C in water bath until methanol is completely evaporated. Subsequently, methanol extracts of those medicinal plants were dissolved in dimethyl sulfoxide (DMSO (10% w/v) at the ratio of 1: 5 (Mishra and Padhy, 2013)to arrive at final concentration of 50 µg/µl. The diluted extracts were stored at 4°C till further usage for the work.

Isolation of organisms

A total of 30 duck feacal samples were collected from Ferreargunj and Namunaghar of South Andaman. The samples were inoculated in nutrient broth and incubated at 37°C overnight. The broth culture was streaked on next day to Eosine Methylene Blue (EMB) and MacConkey agar plates and incubated at 37°C overnight. *E. coli* and *Salmonella* isolates were identified based on colonial morphology on specific agar and biochemical tests(HiMedia, Mumbai, India) as per standard method (Cappuccino and Sherman, 2001;Cheesebrough, 2006).

Antibiogram of isolates

A total of 07 antibiotic discs such as Chloramphenicol, Erythromycin, Tetracycline, Ampicillin, Trimethoprin, Gentamicin and Sulfonamides were assayed. The diameter of the zones of inhibition was measured. The antimicrobial susceptibility data was expressed as percentage of the isolates. Multi drug resistance was defined as resistance exhibited to three or more antimicrobials (Tricia et al., 2006).

Herbal antibiogram

Antibiogram of herbal extracts against isolates were performed using conventional Kirby-Bauer's disc diffusion method (Bauer *et al*., 1966) with Mueller–Hinton (MH) agar medium (HiMedia, Mumbai, India). Six mm filter paper discs were prepared and autoclaved (121°C and 15lbpressure) which were soaked with the extracts by dispensing the extracts on the discs up to the maximum absorption capacity of the discs. The concentration of extracts in each disc was calculated. Gentamicin (G-30µg) disc was used as a positive control and DMSO was added in a separate disc as a negative control. The extracts impregnated discs were placed on the plates which were then incubated for 24 h at 37°C. Zone of Inhibition (mm) of each extract was noted against isolates (Growther *et al*., 2012) and was interpreted with reference to Harun*et al*. (2016).

Results and discussion

A total of 15 (50%) isolates of *Salmonella* and 18 (60%) *E.coli* was identified based on their colony characteristics and biochemical profiles. *E.coli* causes septicemia and diarrhea in ducks (Hofstad *et al*., 1984). The disc method of screening for antibiotic resistance used all these antibiotics are commonly used in the treatment of colibacillosis and salmonellosis and are closely related to those used in human medicine. The results (Table 2)

revealed that the isolates have resistance to almost all tested antimicrobial agents at various rates. The mean inhibition zone was higher against *salmonella* isolates (18.14 mm) as compared to *E.coli* isolates (17.15) although it was not significantly differing. The average percent of *E.coli* isolates sensitive to antibiotics was 46.93 and it was 57.3 with *Salmonella* isolates. Both the isolates showed highest percentage sensitivity (90-100 %) towards gentamicin. The order of percentage sensitive isolates towards antibiotics is gentamicin > trimethopri m>ampicillin>chloramphenicol>sulfonamide. Highest percentage of isolates was resistant to erythromycin followed by tetracycline antibiotics.

The mean of 31.38% of the *E.coli*isolates from poultry under various farming systems were producing Extendedspectrum betalactamases and were multiple antimicrobial resistant. The similar findings of the present study were also reported by singh et al. (2012) that *E.coli* isolates of duck were highly sensitive against chloramphenicol and less sensitive against erythromycin. Similar results were also reported by Unno *et al*. (2010) with *E. coli* isolates of duck in South Korea. Adzitey et al. (2012) and Hamza et al. (2019) also opines with the present results that salmonella isolates (70%) were resistant to erythromycin and susceptible (86-90%) to gentamicin and trimethrim antibiotics.

Table 2: Interpretation of zones of inhibition (in mm)

Duck Feacal *E.coli* Isolates.NS				
Antibiotics	Average ZI (mm)	Sensitive (%)	Intermediate (%)	Resistant (%)
Chloramphenicol	16.61±0.53	53.85	23.08	23.08
Erythromycin	11.00±0.84	7.00	9.00	84.61
Tetracycline	20.00±0.57	10.00	25.00	65.00
Ampicillin	14.00±0.62	70.00	20.00	10.00
Trimethoprim	18.75±0.55	72.72	25.00	3.00
Gentamicin	19.11±0.73	90.00	10.00	0.00
Sulfonamides	20.60±1.23	25.00	55.00	20.00
Mean	17.15±0.62	46.93	23.86	29.38
Duck Feacal *Salmonella* Isolates.NS				
Antibiotics	Average ZI (mm)	Sensitive (%)	Intermediate (%)	Resistant (%)
Chloramphenicol	17.10±0.62	55.65	25.26	19.26
Erythromycin	15.4±0.55	10	19	71.35
Tetracycline	20.6±1.26	25	22	53
Ampicillin	16.3±0.61	85	10	5.52
Trimethoprim	19.1±1.43	80.45	12.14	7.82
Gentamicin	19.11±0.96	100	0	0
Sulfonamides	19.4±0.46	45	35	20
Mean	18.14±0.68	57.30	17.62	25.27

Extracts of Tokurotong (*Tabernaemontana crispa)* (957 µg) exhibited significantly highest ($p<0.05$) antibacterial activity (Table 3) with an average ZI of 16.92 mm against *E. coli* isolates and it was 16.6 mm against the *Salmonella* isolates. Our results indicated that methanol extracts of the plant exhibited strong antimicrobial activity (Harun*et al*., 2016) against the microorganisms tested. Plant based antimicrobials are precious sources for the development of new drugs which can be utilized in both medicine and agriculture. Methanolic extracts of leaves of *T.crispa* exhibited strong antimicrobial activity against clinically isolated *Salmonella* and *E.coli* from ducks. Present results are higher as compared to reports Asma bibi et al. (2016) who obtained ZI of 11.9 mm from the ethanol extracts of *T.crispa* against *E.coli.* The phyto-chemical components such as alkaloids, tannins and saponins present in T.crispa might be responsible. However, Vijayachari, 2016 could not obtain any antimicrobial activity with T.crispa against human pathogens.

Table 3: Antibacterial activity of methanolic extracts of medicinal plants

Sl.No	Plant name	Antimicrobial activity against *E.coli*		Antimicrobial activity against *Salmonella spp*	
		Zone of Inhibition (mm)*	Minimum Inhibitory concentration (µg)	Zone of Inhibition (mm)*	Minimum Inhibitory concentration (µg)
1	Tokurotong (*Tabernaemontana crispa) leaf*	15.7±0.23[a]	957 µg	16.7±0.2[a]	587.00
2	DMSO (*Negative control)*		5.0		
3	Gentamicin (30 µg) (*Positive controls)*		21.0		

Data are presented as mean of measurement of zone of inhibition (ZI) of three replicates measured in mm.They have then categorizedZI 0-6 mm: no activity; 7-10 mm: weak inhibition; 11-15 mm: moderate inhibition; more than 16 mm: strongly inhibited (Harun*et al*., 2016).

*Mean±SE having different superscript differ significantly ($p<0.05$)

Conclusion

Antibiotic resistance pattern is emerging in duck farming also. Based on this study, it may be inferred that trimethoprim and chloramphenicol should be the first choice of treatment against avian colibacillosis and salmonellosis in duck. The resistance pattern observed among the isolated *E. coli* and *salmonella* in A&N Islands might be related to the extensive and indiscriminate use of antibiotic in duck in these areas. A more detailed systemic study is required to extrapolate the present results with herbal extracts. However, this study revealed that methanolic extracts of *Tabernaemontana crispa* have anti-bacterial properties against *E.coli* and *Salmonella* isolated from duck feacal samples and could be further explored for their anti-microbial properties.

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