

Antimicrobial Activity of *Typha angustata* Bory and Chaub Inflorescence Against Wound Associated Bacteria

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Abstract:

Microbes are gaining resistance against chemotherapeutic agents (mostly antibiotics). Thus in recent times it has become very essential to search relatively safe, cheap and effective therapeutics from the plant source.

The objective of the current study is to determine the antibacterial activity of *Typha angustata* Bory and Chaub. aqueous crude extract against bacteria isolated from the patients having different types of wound. A total of 50 clinical samples were obtained from patients having a wound, after their consent. Twenty isolates of pure bacterial cultures were detected. *Escherichia* sp. was found to be the predominant agent isolated from the wound infections (30%) followed by *Staphylococcus* spp. (25%), *Streptococcus* spp., (25%), and *Pseudomonas* sp., (20%). Sensitivity of the bacterial isolates were tested against selected antibiotics: CIP- ciprofloxacin; OFX- ofloxacin; CN- gentamycin; P- penicillin; OX-oxacillin; E- erythromycin, PB- polymyxin B LEV- levofloxacin; OFX- ofloxacin ; AMP- ampicillin; and TE-tetracyclin. The antimicrobial activity of *T. angustata* inflorescence crude extract was determined by well diffusion method. The results revealed that inhibition zone for *Staphylococcus* spp isolates were found to be in the range of (13mm-19mm), *Streptococcus* sp isolates (16mm-19mm), *Pseudomonas* sp. (18mm-20mm) and *Escherichia* sp. (16mm-19mm). The Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) values of *T. angustata* inflorescence crude extract against isolated species from wound samples were recorded in the range of (30-120mg/ml).

The results of this study indicate that *T. angustata* has potential to be developed into antibacterial agent against resistant and susceptible bacteria that are mainly responsible for causing wound infections.

Keywords: Clinical isolates, Wound infection, Antibiotic sensitivity, *Typha angustata* inflorescence crude extract, MIC, MBC.

Introduction

The positive manipulation of a wound is to restore its environment and physiology. The primary factors required to restore environments are adequate moisture level, temperature control, pH regulation and bacterial burden control (Rolstad et al., 2012). Till date, strategies that are used to control bacterial burden includes 1) debridement 2) appropriate wound cleansing 3) appropriate infection control precautions 4) use of antimicrobials and 5) use of a moisture retentive dressing.

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Surgical site is the common nosocomial infections in developing countries. Other wound related infections include diabetic foot ulcer infection, burn wound infection, bite wound infection and pressure ulcer infection (Bhalchandra et al., 2018). Gram positive *cocci* such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus* spp. and Gram negative *bacilli* such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Proteus* species are the most common pathogenic bacteria isolated from wounds (Pallavali et al., 2017). Acute inflammatory episodes are associated with erysipelas and cellulitis are common complications of wounds in lymphoedema patients and most infections are caused by group A, C or G *Streptococci* and *Staphylococcus aureus* (Keeley and Riches, 2009).

Microorganisms responsible to cause cellulitis in lymphoedematous limbs and chronic wound infections include *Staphylococci* sp., *Psuedomonas* sp., *Streptococci* sp., etc. (Eagle, 2007). Fungal infections are also common due to the moisture that is formed between skin folds, resulting in skin breakdown, which in turn leads to infection in the macerated regions. Inflammation of the lymphatic system is also seen due to bacterial infection after invasion through skin wounds or abrasions that leads to lymphagitis (Park et al., 2016).

Excessive use of antibiotics causes destruction to human health, environment and ecosystem. It increases the incidence of drug resistant pathogens (Jastaniah, 2014). Thus, antibiotic resistance is a worldwide major problem that is increasing and has implications for mortality, morbidity and health care both in hospitals and in the community (Mill Robertson et al., 2015).

Most of the pathogenic bacteria have ability to obtain the resistance factor to the antimicrobial drugs, thus development of multiple drug resistant bacteria causes failure in the treatment of infectious diseases (Mill Robertson et al., 2015; Bisht et al., 2009). Therefore, it is necessary to search and design the alternative approaches to control resistant bacteria. One of the best possible strategies is the rationale localization of bioactive phytochemicals with antibacterial activity (Jastaniah, 2014; Ushimaru et al., 2007).

Recent estimation indicates that about 80 million people worldwide still depend on plants for their health needs. In developed and under developed countries, rural people rely on herbal medicines for the treatment of various diseases as they are cheaper and believed to have fewer side effects (Steenkamp et al., 2004; Muthu et al., 2006).

Medicinal plants are used for the treatment of infectious diseases and external wound infections (Adetutu et al., 2011). Moreover, these plants have added benefit of reducing many side effects associated with synthetic antimicrobials (Parekh and Chanda, 2007; Landis, 2008). Folkloric medicines are unexplored resource for the discovery and development of potential new medicines. These medicines are used against microbial infections, reducing emergence of resistant microorganism and adverse reactions. Also, medicinal plants offer several advantages in being affordable, accessible and readily available (Ali and Ju, 2001). Several ethnobotanical studies have reported that the endemic plants have been used by many traditional healers for the range of ailments including wound infections. However, the scientific evidence available regarding the antimicrobial activity of *Typha angustata* inflorescence against wound associated pathogens are still lacking (Saha et al 2021). This study, therefore, aimed to test the

antibacterial activity of the aqueous extract of *Typha angustata* inflorescence against wound associated bacteria.

Material and Methods

Plant collection

Fresh inflorescence of *Typha angustata* Bory and Chaub (Family: Typhaceae) were collected in the month of July 2019 from the campus of Uka Tarsadia University, Bardoli, Gujarat, India. The plant was authenticated (Voucher no- SAHA/CGBIBT/019) at Navsari Agriculture University, Navsari, Gujarat. The inflorescences were shade dried for 2-3 weeks and subjected later for extraction.

Extract Preparation

The extraction procedure was performed according to the method described by Handa et al., (2008). Known weight 100g of shade dried inflorescences was extracted with 200 ml of distilled water using Soxhlet apparatus. Extraction was continued for 5-6 refluxing cycles. The extract thus obtained was filtered using Whatman N1 filter paper. The inflorescence crude aqueous extract was concentrated by rota-evaporator at 50-60°C. The extract was stored at 4°C for antibacterial activity assay.

Bacterial samples

Wound samples were collected from patients with different wound infections. Samples were collected after wound surface was cleansed with normal saline. The inner surface of the infected area was gently swabbed. The swabs were immediately transferred into a tube containing nutrient broth media until further investigations (Manikandan and Amsath, 2013).

Identification of bacterial isolates

Identification of isolates was based on colony morphology and biochemical tests (Triple sugar iron test, Methyl red test, Voges Proskauer, Citrate utilization test, Indole test, various sugar fermentation tests). The collected Gram positive and Gram negative isolates were identified according to Bergey's Manual of Systematic Bacteriology (1989) and Cheesebrough (1984).

Preparation of inoculums

The inoculums were standardized to give density of 10⁶ colony- forming units (CFU/ml). For standardizing, a loopful of test organism was inoculated into sterile nutrient broth and incubated at 37°C for 24 hours. 0.2ml from the 24 hour culture of the organism was dispensed into 20ml sterile nutrient broth and incubated for 4-5 hours to obtain 10⁶ CFU/ml (corresponding to 0.5 McFarland standards). To avoid any changes in the inoculum's density, plates were inoculated within 15 minutes of standardizing the inoculums (Abalaka et al., 2012).

Antibiotic sensitivity test

The isolated bacterial species were assessed for the sensitivities against different antibiotics based on disc diffusion (Kirby-Bauer) technique (Bauer et al., 1966) as described by Saif et al. (2017).

Antibacterial assay

Antibacterial assay was carried out by agar well diffusion method as described by Das

et al. (2013). 100 µl of standardized inoculum of fresh microbial culture was spread on Muller Hilton agar plate. Wells were punched off into the agar medium with sterile cork borer. Under aseptic condition, well was filled with 100µl of *T. angustata* inflorescence aqueous crude extract at concentration (500µg/ml). DMSO was used as a negative control. After 1 hour, plates were incubated in an upright position at 37°C for 24 hours. The screening for antibacterial activity was evaluated by measuring zone of inhibition in (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The lowest concentration of the drug which will inhibit growth i.e. minimum inhibitory concentration of the drug was measured by observed turbidity in the test tube (CLSI, 2016). The MIC of the inflorescence aqueous crude extract was determined using methods described by Usman et al. (2014) and Wiegand et al., (2008). Serial dilution of the extract was done to obtain concentrations of 7.5µg/ml, 15µg/ml, 30µg/ml, 60µg/ml, 120µg/ml, 240µg/ml and 480 µg/ml. 100 µl of standardized culture of tested organisms were inoculated into each tube containing nutrient broth and plant extract and incubated at 37°C for 24 hours. Tube containing nutrient broth inoculated with bacteria was used as positive control.

Results and Discussion

Tissue repair that are results of cellular response is ordered with regulated intercellular communication. Tissue repair is frequently depicted as consecutive, congruous phases: haemostasis, inflammation, proliferation and remodelling. Deregulation of this ordered cellular event results into impaired healing, leads to manifestation of chronic non healing wounds, tumor formation and excessive scarring (Goldberg and Diegelmann, 2010). Deregulation is generally caused due to microbial invasion and repetitive injury.

From 50 specimens collected, total 20 (40%) yielded pure bacterial culture. The bacterial isolates from the wounds were as follows: 14 (70%) isolates were Gram positive and 6 (30%) isolates were Gram negative. The detected organisms were 5 (25%) *Staphylococcus* spp., 5 (25%) *Streptococcus* spp., 4 (20%) *Pseudomonas* sp., 6 (30%) *Escherichia* sp. Our findings correlate with (Zhang et al., 2014) who reported predominance of *E. coli*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* in pus samples from patients with severe intra-abdominal infection. Report of Bessa et al. (2015) states that *S. aureus* was the dominant bacterial species from wound followed by *P. aeruginosa*, *P. mirabilis*, *E. coli* and *Corynebacterium* spp. Several reports (Misic et al., 2014; Lockhart et al., 2004) have also implicated *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, and *E. coli* in wound infections.

Staphylococcus spp. showed high sensitivity to ofloxacin, gentamycin, oxacillin and erythromycin (100%) (Table -1), in the same table, *Staphylococcus* spp. isolates St. 1,3,5 were (resistant) to penicillin. Isolate St.4 showed intermediate sensitivity against ciprofloxacin. Whereas, isolates St.2 and St. 5 were resistant to ciprofloxacin. *Streptococcus* spp showed high sensitivity to ofloxacin, erythromycin (100%), Isolate Sa. 2 was resistant to penicillin and was intermediate to ciprofloxacin. Isolate Sa.3 was resistant to penicillin and gentamycin.

Table1: Susceptibility test of clinical isolates from different type of wounds belonging to genus *Staphylococcus* and *Streptococcus* against standard antibiotics

Bacterial Sam-ples	CIP	OFX	CN	P	OX	E
<i>Staphylococcus</i> sp.						
St.1	S	S	S	R	S	S
St.2	R	S	S	S	S	S
St.3	S	S	S	R	S	S
St.4	I	S	S	S	S	S
St.5	R	S	S	R	S	S
<i>Streptococcus</i> sp.						
Sa.1	S	S	S	S	S	S
Sa.2	I	S	S	R	I	S
Sa.3	S	S	R	R	S	S
Sa.4	S	S	S	S	S	S
Sa.5	S	S	S	S	S	S

The diameters of the inhibition zones were interpreted according to CLSI (2016), The isolates were reported as R- resistant, I- intermediate and S- Sensitive. Where CIP- ciprofloxacin; OFX- ofloxacin; CN- gentamycin; P- penicillin; OX-oxacillin; E- erythromycin *Pseudomonas* sp. showed 100% sensitivity against ciprofloxacin, moderate sensitivity against levofloxacin and polymyxin B (92.3%); 50% isolates showed resistance to ofloxacin shown in (Table-2). The individual members of fluoroquinolones demonstrate different spectra of activity and pharmacokinetic profiles, DNA gyrase (topoisomerase II) and DNA topoisomerase IV (Aldred et al. 2014). These drugs trap a reaction intermediate containing quinolone enzyme and broken DNA, that leads to the blockage of DNA replication. While for some bacteria death occurs within hours. In addition, polymyxins binds to the cell membrane and alter its structure. This results into an increase permeability of the cell envelop consisting of the cell wall and cytoplasmic membrane. Subsequently, cell death occurs due to leakage of cell contents (Parija, 2012). Hooper and Jacoby (2015) reported that resistance is due to changes in DNA gyrase enzyme and/or the topoisomerase enzymes or by the defective function of porine channels. Saha et al. (2017) and Bhalchandra et al. (2018) reported that *P. aeruginosa* strains were sensitive to levofloxacin, polymyxinb and ciprofloxacin and showed high resistance against ceftazidme and aztreonam.

Table 2: Susceptibility test of clinical isolates from different type of wounds belonging to genus *Psuedomonas* against standard antibiotics

Bacterial Sam-ples	CIP	LEV	OFX	PB
<i>Psuedomonas</i> sp.				
P.1	S	S	S	S
P.2	S	S	S	R
P.3	S	R	R	S
P.4	S	S	R	S

The diameters of the inhibition zones were interpreted according to CLSI (2016), The isolates were reported as R- resistant, I- intermediate and S- Sensitive. Where CIP- ciprofloxacin; LEV- levofloxacin; OF- ofloxacin; PB- polymyxin B.

Escherichia sp. isolates showed 100% sensitivity against ampicillin and tetracycline. Few isolates were variant (resistant or sensitive) to levofloxacin. Few isolates were variant (intermediate or sensitive) to ofloxacin. *Escherichia coli* isolates sensitivity against levofloxacin was found to be (92.3%) as shown in (Table-3). Similar observations have been reported by Yakha et al.(2015); Gomatheswari and Jeyamurugan (2017), and Saha et al.(2017) that imipenem, amikacin, and gentamycin were very effective drugs against *E. coli* isolates but presented resistance against cefuroxime, ciprofloxacin, ofloxacin, cefotaxime, and ampicillin.

Table 3: Susceptibility test of clinical isolates from different type of wounds belonging to genus *Escherichia* against standard antibiotics

Bacterial Sam-ples	CIP	LEV	OFX	AMP	TE
<i>Escherichia</i> sp.					
E.1	S	S	S	S	S
E.2	S	S	I	S	S
E.3	S	R	S	S	S
E.4	S	S	I	S	S
E.5	S	S	S	S	S
E.6	S	R	S	S	S

The diameters of the inhibition zones were interpreted according to CLSI (2016), The isolates were reported as R- resistant, I- intermediate and S- Sensitive. Where CIP- ciprofloxacin; LEV-levofloxacin; OFX- ofloxacin ; AMP- ampicillin; TE-tetracyclin.

The aqueous crude extract of *T. angustata* inflorescence represented antibacterial activity against most of the sensitive and resistant isolates (Table-4). The diameter of zone of inhibition for *Staphylococcus* spp isolates were found to be in the range of (13mm-19mm), *Streptococcus* sp isolates were found to be in the range of (16mm-19mm), *Psuedomonas* sp. isolates were found to be in (15mm-20mm). *Escherichia* sp. isolates were found to be in (15mm-19mm). The anti-microbial activity exhibited by the *T.angustata* inflorescence aqueous crude extract might be attributed to its broad-spectrum nature. High MIC was recorded for *Staphylococcus* spp., (St. 1,2), *Streptococcus* sp. (Sa.5), *Escherichia* sp. (E3) with 60 µg/ml, MBC found to be 120 µg/ml. Lower MIC values were observed for *Staphylococcus* spp.(St. 3,4,5) *Streptococcus* sp. (Sa.1,2,3,4). *Psuedomonas* sp. (P.1,2,3,4) and *Escherichia* sp. (E.1,2,4,5,6) with 30 µg/ml. Whereas, MBC found to be 60 µg/ml (Table-5).

Table 4: Antimicrobial activity in(mm)of *T. angustata* inflorescence aqueous crude extract (in mm) against isolates from wound.

Bacterial Isolates	Diameter of inhibition zone in mm
<i>Staphylococcus</i> sp.	19
St.1	16
St.2	13
St.3	19
St.4	19
St.5	19
<i>Streptococcus</i> sp.	19
Sa.1	18

Sa.2	17
Sa.3	19
Sa.4	19
Sa.5	16
<i>Psuedomonas sp.</i>	19
P.1	18
P.2	19
P.3	20
P.4	18
<i>Escherichia sp.</i>	18
E.1	18
E.2	17
E.3	16
E.4	17
E.5	18
E.6	19

Table 5: Determination of MIC and MBC of *Typha angustata* inflorescence aqueous crude extract against Clinical Isolates

Concentrations of <i>T. angustata</i> L. inflorescence aqueous extract in µg/ml								MIC µg/ml	MBC µg/ml
Bacterial Isolates	7.5	15	30	60	120	240	480		
<i>Staphylococcus sp.</i>									
St.1	+	+	+	+	-	-	-	60	120
St.2	+	+	+	+	-	-	-	60	120
St.3	+	+	+	-	-	-	-	30	60
St.4	+	+	+	-	-	-	-	30	60
St.5	+	+	+	-	-	-	-	30	60
<i>Streptococcus sp.</i>									
Sa.1	+	+	+	-	-	-	-	30	60
Sa.2	+	+	+	-	-	-	-	30	60
Sa.3	+	+	+	-	-	-	-	30	60
Sa.4	+	+	+	-	-	-	-	30	60
Sa.5	+	+	+	+	-	-	-	60	120
<i>Psuedomonassp.</i>									
P.1	+	+	+	-	-	-	-	30	60
P.2	+	+	+	-	-	-	-	30	60
P.3	+	+	+	-	-	-	-	30	60
P.4	+	+	+	-	-	-	-	30	60
<i>Escherichia sp.</i>									
E.1	+	+	+	-	-	-	-	30	60
E.2	+	+	+	-	-	-	-	30	60
E.3	+	+	+	+	-	-	-	60	120
E.4	+	+	+	-	-	-	-	30	60
E.5	+	+	+	-	-	-	-	30	60
E.6	+	+	+	-	-	-	-	30	60

Where, MIC minimum inhibition concentration, MBC minimum bacterial concentration, + showing growth, - showing no growth.

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of antimicrobial agent that inhibits the visible growth of microorganisms after overnight incubation. The MBC is complementary to the MIC. It demonstrates that lowest level of antimicrobial agent that results in microbial death after sub culturing the organism in an antibiotic free media (Owuama, 2017). The MIC is used to evaluate the antimicrobial effectiveness of new compounds or plant extracts by measuring the effect of decreasing the antimicrobial concentration. Antimicrobial agents with lower MIC are considered to be more effective. The MIC values obtained from the present study indicates that aqueous extract of *Typha angustata* Bory and Chaub inflorescence was more potent against *Staphylococcus* spp., (St. 3,4,5) *Streptococcus* sp. (Sa.1,2,3,4). *Psuedomonas* sp. (P.1,2,3,4) and *Escherichia* sp. (E.1,2,4,5,6), which agrees with the initial antimicrobial screening test results (agar well diffusion test). The results of our study are in linewith those of previous report from Jordan (Kouadri, 2018). The differences in bacterial susceptibility could be due to variations in intrinsic tolerance of micro-organisms, or the physio-chemical properties of phytochemicals present in the crude extracts of the plant materials (Kouadri, 2018). The MBC/MIC ratio was determined for *T. angustata* aqueous crude extract was bactericidal or bacteriostatic at the tested concentrations. The MBC/MIC ratio greater than 4 is usually considered to be a bacteriostatic effect; whereas values less than 4 show bactericidal effects; (Venkateswarulu, 2019). Accordingly, *T. angustata* aqueous extract was shown to have bactericidal effects against studied wound isolates.

Conclusion

The present study revealed that *Staphylococcus* sp., *Streptococcus* sp., *Psuedomonas* sp., *Escherichia* sp., were the most common isolates that are associated with wound. The susceptibility test of clinical isolates was carried out against standard antibiotics like ofloxacin, gentamycin, penicillin, oxacillin, erythromycin, levofloxacin, polymyxin B etc. The susceptibility tests confirmed several sensitive, intermediate and resistant strains. These isolates when exposed to different concentrations of aqueous crude extract. The findings of current study revealed that aqueous extract from the inflorescences of *Typha angustata* Bory and Chaub has good potential as a antimicrobial agent. However, further study need to be conducted. In addition, probable mechanisms of the possible antimicrobial activity should also be studied against wound associated bacteria.

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نشاط مضادات الميكروبات من إزهار *Typha angustata* ضد البكتيريا المصاحبة للجروح Bory and Chaub

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المُستخلص

تكتسب الميكروبات مقاومة ضد عوامل العلاج الكيميائي (معظمها من المضادات الحيوية). وهكذا أصبح من الضروري في الآونة الأخيرة البحث عن علاجات آمنة ورخيصة وفعالة نسبياً من مصدر النبات.

الهدف من الدراسة الحالية هو تحديد النشاط المضاد للبكتيريا لـ *Typha angustata* Bory and Chaub. مستخلص خام مائي ضد البكتيريا المعزولة من المرضى الذين يعانون من أنواع مختلفة من الجروح. تم الحصول على ما مجموعه 50 عينة سريرية من المرضى الذين يعانون من الجروح، بعد موافقتهم. تم الكشف عن عشرين عزلة من الثقافات البكتيرية النقية. *Escherichia sp.* وجد أنه العامل السائد المعزول من التهابات الجروح (30%) يليه المكورات العنقودية. (25%)، المكورات العقدية، (25%)، و (20%) *Pseudomonas sp.* تم اختبار حساسية البكتيريا المعزولة ضد مضادات حيوية مختارة: CIP- سيبروفلوكساسين. CN ، ofloxacin - OFX- جنتاميسين. ف- البنسلين. E- OX-oxacillin. الإريثروميسين ، PB- بوليميكسين B-LEV- ليفوفلوكساسين ؛ ofloxacin - OFX ؛ AMP- الأمبيسلينسي. تم تحديد النشاط المضاد للميكروبات لمستخلص إزهار *T. angustata* الخام بطريقة نشر البئر. كشفت النتائج أن منطقة تثبيط المكورات العنقودية المعزولة spp كانت في نطاق (13 مم - 19 مم)، المكورات العقدية sp المعزولة (16 مم - 19 مم) ، (18 مم *Pseudomonas sp.* 20- مم) و (16 *Escherichia sp.* 19- مم). تم تسجيل قيم الحد الأدنى للتركيز المثبط (MIC) والحد الأدنى للتركيز البكتيري (MBC) لمستخلص *T. angustata* الإزهار الخام ضد الأنواع المعزولة من عينات الجرح في نطاق (30-120 مجم / مل).

تشير نتائج هذه الدراسة إلى أن *T. angustata* يمكن تطويره ليصبح عاملاً مضاداً للبكتيريا ضد البكتيريا المقاومة والحساسية المسؤولة بشكل رئيسي عن التسبب في التهابات الجروح.

مفاتيح الكلمات: العزلات السريرية ، عدوى الجرح، حساسية المضادات الحيوية، مستخلص الخام الإزهار Bory and Chaub *Typha angustata* ، MIC ، MBC.

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