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Research Article

Herbal Therapy Against Pulmonary Diseases In Pig And Man With *Typhonium trilobatum* Schott (L.) Schott. An Indian Medicinal Plant

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ABSTRACT

Respiratory tract infections are common ailments in man and pig. Several species of bacteria are associated with the infection. Considering the uses of *Typhonium trilobatum*(L.) in both Unani and Ayurvedic medicinal systems, we approached to investigate its efficacy against selected respiratory tract bacterial pathogens associated with both man and pig. The tuber extract was prepared using hydro-ethanol (80:20). Different phytochemicals present were characterized based on GC-MS and NMR. Bacteria species were isolated and identified from the nasal swabs and tissues samples of patients and pigs suffering from respiratory disease. The antibacterial activity of the extract was evaluated using agar well diffusion and micro-broth dilution method to determine the MIC and MBC. Further, the efficacy of the extract was tested by monitoring the complete recovery of the pigs suffering from respiratory disease. Hydro-ethanolic extracts of *T. trilobatum* showed significant antibacterial activity with the bacterial flora isolated from both the patients and pigs. Experimental animals suffering from flu-like symptoms recovered in 3-6 days with oral supplements of 500-300 mg/mL/day/pig. The antibacterial efficacy of the hydro-ethanolic extract can be attributed to the presence of three broad groups of compounds such as organic acids, monosaccharides and polyols as detected by GC-MS. This study validated the antibacterial properties of *T. trilobatum* tuber extract against bacterial flora isolated from patients and pigs causing respiratory tract infections. Also, the animals were found free from flu-like symptoms without any adverse reaction with the oral supplement of the extract.

Keywords: *Typhonium trilobatum*, pulmonary diseases, multidrug-resistant bacteria, GC-MS, NMR, *In vitro* and *In vivo* studies.

INTRODUCTION

Respiratory tract infections in man are common ailments and different species of bacteria and viruses are reportedly associated with the infections. Several antibiotics have been used indiscriminately which lead to the development of multi-drug resistance (MDR) by many bacteria. Presently microbial resistance to antibiotics is considered to be a major threat to human health and the environment. Literatures projected, by the end of 2050, around 10 million deaths will occur due to multidrug-resistant (MDR) bacteria. Also, the development of novel antibiotics is a slow process.[1] Further, currently used antibiotics are mostly broad spectrum in nature and the use of such antibiotics leads to indiscriminate killing of beneficial commensal bacteria that may lead to the evolution of drug-resistant bacterial flora. Drug resistance is one of the most noteworthy clinical issues that have to be addressed for the wellbeing of human health.

Respiratory diseases also have a significant impact and are responsible for losses in the productivity of pig herds.[2] Various infectious

agents such as virus, bacteria and mycoplasma are associated with respiratory infections in the pig.[3] Among the bacterial species *Pasteurella multocida*, *Streptococcus suis* and *Staphylococcus aureus*, etc. are commonly found in the respiratory tract infection in the pig. Indiscriminate use of antimicrobials as growth promoters and in the controlling of bacterial infections has resulted in the emergence of MDR bacteria which not only complicates the treatment but also increases the cost of treatment. Besides, antibiotic-resistant bacteria can be transferred from pigs to man particularly to the pig bearers and pork consumers. Besides, resistant bacteria which are excreted from a pig can contaminate the surrounding environment, both soil & water.[2,3]

Out of several ways to reduce a load of antibiotic-resistant bacteria in the environment, purified and partially purified compounds from the medicinal plants, seem to be very promising in the treatment of bacterial infections[4]. Considering the efficacy of *Typhonium trilobatum*(L.) Schott, against various systemic and infections diseases, [5-8] the present study was undertaken to determine its efficacy against selected MDR bacteria causing of man and pig. Specifically, the pig is included in the animal study because they are an ideal model for biomedical research in man due to the similarity in anatomical size and structure of the internal organs.[9] Besides, most of the bacteria associated with respiratory tract infections in pig are similar to respiratory tract infections in man.

Typhonium trilobatum (L.) Schott is a perennial herb belonging to the family Araceae, distributed throughout the tropical and subtropical regions of the world with greater concentration in Southeast Asia particularly in India and Bangladesh, Indo-Malaysia and North-eastern Australia.[10,11] The genus *Typhonium* is comprised of about 30 species and in India,[12] its is represented by 16 species.[13] Among the species found in India, *T. trilobatum* (L.) Schott is the most common and grows wild in a wide range of habitats. This plant is a proven medicinal plant as it's used for both systemic and infectious disease, which has been reported in many scientific, ayurvedic and Unani medicinal literature.[14-17]

MATERIALS AND METHODS

Collection of plant material and Preparations of plant fractions

Mature and dormant corms of *T. trilobatum* were collected from the medicinal plants garden of Odisha University of Agriculture and Technology, Bhubaneswar. Corms were washed thoroughly under tap water for removal of root, dirt, etc., followed by washing in distilled water, cut into 1 cm^3 and dried to constant weight in a hot air oven at a temperature of 60ºC and stored for further use. The dried samples were made into powder using a pulverizer and used for hot solvent extraction was done using hydro-ethanol (40:60) in a soxhlet apparatus for 24 h. The extract was dried using a rotary evaporator at 60ºC for 4 h and stored at -20ºC for use in subsequent experiments. The voucher specimen was deposited in the SFTRRD herbaria.

Phytochemical profiling of crude extract

The extraction of metabolites from the dried extract was done by the method described by Roessner et al. (2003)[18] and Rangani J et al. (2019)[19]. As per the published protocol " the sample was extracted in 1.4 mL of methanol and ethanol using 100 μL of ribitol (1 mg mL^{-1}) as an internal standard. The extract was mixed properly and incubated for 15 min at 70ºC with shaking (200 rpm). After incubation, an equal amount of water and chloroform (750 μL) was added and mixed vigorously. The mixture was centrifuged at 22,000 $\times$ g at room temperature for 15 min. 200 μL of this supernatant was transferred into another tube and vacuum dried for further derivatization process." [18, 19]

For GC/MS analysis, methods described by Roessner et al. (2003)[18] and Rangani J et al. (2019)[19] was followed where "2 µL of the sample was injected in the GC column connected with the GC/MS system (GC/MS-QP2010, Shimadzu, Kyoto, Japan). The separation of the derivatized compounds was performed on an SH-Rxi-5 ms column (30 m, 0.25 µm df, Shimadzu, USA) with split injection mode and the injector temperature was maintained at 250ºC. Helium was used as the carrier gas with a flow rate of 1 $mL.min^{-1}$. The ion source was tuned to 250ºC and the transfer line was set at 300ºC with the rate of $14.5ºC.s^{-1}$. The mass spectra were recorded at a rate of eight scans per second with a scanning range of 70-700 m/z."[18, 19]

Identification of compounds

Identification of the compounds was done by the method mentioned in Dubey et al. (2014)[20], a previous paper published by one of our co-authors. As per the published protocol "the phytochemical components of the biologically active fraction, the hydro-ethanolic and methanolic fraction were identified by comparison of their mass spectra fragmentations and retention indices with those stored in databases, NIST08.LIB (Stein SE National Institute of Standards and Technology, Mass Spectral Database and Software, Version 3.02, USA, 1990) and WILEY8.LIB (McLafferly FW Registry of

mass spectral data, ed. 5, Wiley New York, 1989), and also with the published literature."[20]

^{1}H-NMR spectroscopic analysis

The dried extract was dissolved in deuterated chloroform ($CDCl_3$, D: 99.8%, Merck) containing 0.01% trimethylsilyl propionic acid-d4 sodium salt (TSP-d4, Merck) serving as internal standard, and filtered through a 0.2 μm syringe filter. About 600 μL of the resulting solution was loaded into the NMR tube for analysis. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) experiment was performed on Bruker Avance III 600 MHz spectrometer (BrukerBiospin GmbH, Reinsteten, Germany), operating at 600.17 MHz at 300 K, with 900 pulse widths at 5.75 sec, relaxation delay of 1 sec, 16 scans, 64 K data point and a spectral width of 17023 Hz. The raw data was acquired in the form of free induction decay (FID) using Topspin 2.1 software for Bruker Avance systems.

Sample collection and isolation of bacteria from pigs

Nasal swabs and tissue samples from pigs affected with respiratory tract infection were collected from different pig farms of Guwahati, Lakhimpur and Nalbari District of Assam. The samples were collected aseptically and immediately brought to the laboratory for further downstream processing. Samples were cultured on suitable specialized agar media and identified using standard microbiological and biochemical methods. American type culture collection (ATCC) standard strains of bacteria were used as reference controls. Two Gram positives (GPs), *S. aureus* , *S. suis* and two-gram negative (GN) bacteria *P. multocida* and *E. coli* were isolated and used in this study (Table 1).

In vitro studies on the plant extract against bacteria isolated from pigs

Macrodilution method

The antimicrobial activity of the plant extract was studied *In vitro* using the macro-dilution method (NCCLS, 2000). Four different concentrations of the plant extract of viz. 50, 100, 200 and 300 mg/mL were prepared from the stock solution. One millilitre of a broth culture of bacterial strains containing approximately $1x10^8$ CFU/mL was transferred to separate sterile tubes containing the plant extract at each of the concentrations i.e.50-300 mg/mL and incubated at 37ºC for 24 h. Commercially available biotrim DS tablet @ 200 mg /mL was used as a control against each of the bacterial strains.

Disc diffusion method

To compare the antibacterial activity of the plant extract and other commercially available broad-spectrum antibiotics, the disc diffusion method was performed (NCCLS, 2000). Briefly, sterile discs impregnated with four concentrations i.e. 100 mg/mL, 200 mg/mL, 300 mg/mL and 500 mg/mL of the plant extract was dried in the incubator and used in the test along with the commercially available three antibiotic discs and biotrim DS disc of 500 mg/mL. Broth culture of the four bacterial suspensions containing approximately $1x10^8$ CFU/mL was uniformLy inoculated on Mueller Hinton agar plates and the plates were then allowed to dry for five minutes. The sterile disc of 6 mm was soaked in 10 μL of plant extract and then the discs containing the plant extract were placed on the inoculated Muller Hinton agar plates along with the commercially available antibiotic discs namely amikacin, ceftriaxone and tetracycline antibiotic discs and biotrim DS disc were placed and incubated for 24 h at 37ºC. After incubation, the zone of inhibition surrounding the discs was measured using the Hi zone antibiotic scale; (Hi-Media Laboratories Pvt. Ltd., Mumbai, India).

Minimum Inhibitory Concentration (MIC)

MIC of the plant extract was used by using the microdilution method. Fivefold serial dilution of the plant extract was prepared in 10% DMSO to obtain seven serial dilutions of the original extract. Inoculum of each organism was prepared in Mueller Hinton broth and the turbidity was adjusted to approximately 0.5 McFarland turbidity standard to prepare $1X10^8$ bacterial/mL. In a 96 well microtitre plate, 150 μL of plant extract was added to each well and 50 μL of bacterial suspension was added to each well except the negative controls. Plant extract in 200 μL of 10% DMSO without bacterial suspension was used as a negative control. Ceftriaxone was used as a positive control. The Microtitre plate was incubated at 37ºC for 24 h. Antimicrobial activity was assessed by measuring absorbance at 630 nm wavelength.

In-vivo efficacy testing in pig

To determine the *In vivo* antibacterial activity of the extract, five groups (A-E) of pig having 5 numbers in each group and showing clinical signs of respiratory tract infection were selected. The average bodyweight of the selected animals in each of the group was 17 kg and about 2 months old. Crossbred animals of this age group showed more severe respiratory infections, hence selected for the study. Animals of group A were given the plant extract @ 500 mg/animal/day and group C

was given the extract @ 300 mg/animal/day for three days to five days orally. Animals of the group B and D were given the biotrim Ds @ 500 mg/animal/day and 300 mg/animal/day orally for three to five days respectively. Animals of group E were kept as untreated control for five days. All the groups were kept under full supervision provided with daily maintenance ration. Nasal swabs from each of the pigs were taken to isolate the bacteria involved in the respiratory tract infection before and after the treatment.

Isolation and identification of bacteria from the clinical samples of patients

Samples like throat swabs, nasal swabs, nasal aspirates, and nasal wash from the patients suffering from respiratory disease admitted to IMS and SUM Hospital, Odisha, India, were collected. The bacterial species from the samples were isolated and cultured on suitable agar media. The pure cultures of the isolated bacteria were identified using VITEK2 (Biomereux) and standard biochemical procedures.[4, 21]

Antibiotic susceptibility test

All the 3 bacterial strains, *S. aureus* , S. *pyogenes* and *K. pneumoniae* isolated from the clinical samples, including the standard MTCC strains of each bacterium were subjected to antibiotic sensitivity following Clinical Laboratory Standard Institute (CLSI) guidelines.[22,23.]

Detection of MRSA by chromogenic agar media test

S. aureus was categorized as MRSA and non-MRSA using Hichrome-MeReSa agar media, Himedia as described earlier.[4]

Detection of ESBL producers by double-disc synergy test

GN bacteria were subjected to a double-disc diffusion test for confirming their ESBL producing capacity as described earlier.[24]

Antibacterial activity of the plant extract

Antibacterial activity of the plant extract was done by the agar well diffusion method.[23] Linezolid 30 μg/mL (for GP bacteria), imipenem 10 μg/mL (for GN bacteria) and 10% DMSO were taken as positive and negative reference controls, respectively.

Determinations of MIC and MBC

MIC and MBC values of plant extracts were determined using the microbroth dilution method.

RESULTS AND DISCUSSION

Chemical profiling of aqueous ethanolic extract of the tuber

The hydro-ethanolic extract of mature and dormant corms of *T. trilobatum* was used for chemical profiling using GC-MS and NMR techniques. Since the hydro-ethanolic extracts of mature and dormant corms of *T. trilobatum* do not contain any volatile components, we have derivatized with silylating agents (vide infra) and then analysed them with GC-MS (Fig. 1). Broadly, three groups of compounds found in the extract such as organic acids, monosaccharides and polyols (Table 2). Ten organic acids were found with a total composition of 50.54% of the extract. There existed one aromatic acid (0.88%), one amino acid (0.15%), four long-chain alkanoic acids (46.59%) and two dicarboxylic acids (2.23%). It was also found to contain myoinositol (1.29%) and four monosaccharides (26.28%). Four esters of long-chain alkanoic acids (13.26%) and one amide of 9-Octadecenoic acid were also detected in the extract. In addition to these, glycerol (5.55%) and monoglyceryl ester of palmitic acid (0.63%) were also present as detected by their mass spectrum. The major group of compounds of the extract are organic acids. Among them, four long-chain alkanoic acids constituted 46.59% of the extract. A major component of the extract is palmitic acid (29.14%) and oleic acid (13.84%). The biological activities of the aqueous ethanol extract are probably due to the presence of these four organic acids.[25] Lactic acid and benzoic acid also have a synergistic effect with other organic acids contributing to these activities. Glycidyl ester of oleic acid may also contribute towards the antibacterial activity of the extract as it contains an epoxide moiety. Epoxides are active components of organic compounds usually due to their strained three-membered rings. Further, the monosaccharide, D-Psicofuranose was also found to occur in 18.82% of the extract.

We have also obtained another bioactive fraction of *T. trilobatum* rhizomes (underground) using aqueous methanol which showed anti-inflammatory activity. The GC-MS analysis of the methanolic extracts leads to the identification of several compounds. These compounds were identified through mass spectroscopy attached with gas chromatography. The various compounds present in the entire rhizome of *T. trilobatum* are shown in Table 3. The composition determined for the methanolic extracts corresponds to 87% of the entire GC-MS chromatogram. The GC-MS spectrum confirmed the presence of various components with a different retention time as illustrated in

supplementary Fig. S1-S4. The mass spectrometer analyses the compounds eluted at different times to identify the nature and structure of compounds (supplementary Fig. S5). The compound fragments into small compounds giving rise to the appearance of peaks at different m/z ratios. These mass spectra are the fingerprint of that compound which can be identified from the data library.[26]

^{1}H-NMR spectrometry analysis was performed to evaluate the lipid profiling from methanolic extracts of *T. trilobatum* tubers. Before peak assignment, NMR spectra were phased, baseline corrected and normal smoothing done manually in Mestronova. The ^{1}H-NMR (Fig. 2) chemical shift was referenced to TSP signal at δ 0.00 ppm. Acquired spectra were normalized with the peak intensity of TMS at 0.00 ppm. Overlapped signals were identified by the 2D-NMR, Correlation spectroscopy (^{1}H-^{1}H COSY) & Total correlation spectroscopy (TOCSY) NMR (Supplementary Fig. S6 and S7) and integrated manually. The ^{1}H NMR data (Table 4) revealed the presence of a trans-double bond (δH 5.44, dd, J = 15.2, 6.8; δH 5.62, dd, J = 15.2, 6.8, 4.4 Hz) and hydroxy group (δH 4.03, q, J = 6.4 Hz) in long-chain fatty acid, which was methylated (δH 3.656, s). The position of the double bond and hydroxy group was assigned by GC-MS, which showed a strong peak at m/z154 ($C_9H_{14}O_2$) and was identical to the reported spectroscopic data.[27] The present study helps to predict the formula and structure of 49biomolecules from methanolic extract of *T. trilobatum* (Supplementary Fig. 5). Further investigation may lead to the isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be helpful for further drug development.

Plant extract effectively inhibits the growth of isolated bacteria from pig

The results of the antibacterial activity of the plant extract at four different concentrations against isolated two GN bacteria viz. *P. multocida* and *E. coli* as well as two GP bacteria viz. *S. suis* and *S. aureus* from pigs suffering from respiratory tract infection are presented in Table 5. The plant extract at lower concentrations viz. 50 and 100 mg/mL is unable to inhibit the growth of all four bacteria. In contrast, a higher concentration of 200 and 300 mg/mL of the plant extract could effectively inhibit the growth of the bacteria as indicated by the absence of turbidity in the tubes. Similarly, the biotrim DS @200 mg/mL inhibited the growth of the bacteria.

Based on the sensitivity of the broth dilution assay four concentrations (100 mg/mL, 200 mg/mL, 300 mg/mL and 500 mg/mL) of plant extracts were used in the present study. The zone of inhibition ranges from 7mm to 24 mm with the treatment of plant extract among four bacterial species isolated from pigs (Table 6). Although all the three broad-spectrum antibiotics viz. tetracycline, ceftriaxone and amikacin showed a higher zone of inhibition (Table 6) against the bacteria than the zone of inhibition produced by the plant extract @ 300 mg/mL and 500 mg/mL, but improper use of these antibiotics usually have more chances of developing resistant bacteria. As an example the isolated *Pasteurella multocida* from the pigs although showed sensitivity towards tetracycline, ceftriaxone and amikacin, it has reported earlier to showed100% resistance to Ceftizoxim and Cloxacillin.[28] Similarly, *S. suis* isolates from pigs showed multidrug resistance including tetracycline has already been reported from Assam, India.[29]. Another emerging concern is the development of methicillin-resistant of *S. aureus* in animal, primarily pig in many countries.[30] These resistant bacteria, particularly *S. suis* and *S. aureus* from pigs may have a significant impact on human health. Hence the plant extract which showed inhibition of growth and zone of inhibition of the bacteria at 300 and 500 mg/mL could be the choice of treatment of respiratory infections in pig mostly associated with the bacterial species. The presence of these bacteria in the respiratory tract infections of pigs or as a secondary invader to certain virus infections has been reported by other workers.[31]

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration

The antibacterial activity of the plant extracts was further determined using a microdilution method. The MIC values and MBC values of different antibiotics and plant extract for different bacteria isolated from pig are presented in Table 7. Surprisingly all the isolated bacteria were susceptible to plant extract albeit at a higher concentration than standard antibiotics. The MIC value of plant extract is 5.25 mg/mL, 6.25 mg/mL, 10.25 mg/mL and 14.5 mg/mL with *S. aureus* , *S. suis*, *P. multocida* and *E. coli*, respectively. Parenthetically, the MBC value of plant extract is 21 mg/mL, 31.25 mg/mL, 41.0 mg/mL and 58.0 mg/mL with *S. aureus* , *S. suis*, *P. multocida* and *E. coli*, respectively. The MIC and MBC value of the plant extract was compared with four other antibiotics. In the present study, the T. rilobatum tuber extract exhibited significant antimicrobial activity against Gram-positive as well as to the Gram-negative bacteria.

In vivo evaluation of the plant extract showing a recovery in respiratory diseases of pig

The recoveries of animals from the respiratory infection with the oral gavage of hydro-ethanolic extract of *Typhonium trilobatum* are collated in Table 8 and Fig. 3. None of the animals showed any adverse effect at daily doses of 500 mg/mL. The group of animals that received the plant extract @500 mg/mL showed recovery from the respiratory tract illness within 3 days, whereas the animals which received the plant extract @300 mg/mL recovered in 5-6 days. On the other hand, the animals which received biotrim DS @500 mg/mL showed recovery in 3-4 days. The nasal swabs which were collected from the pigs showing respiratory distress and before the treatment yielded mixed infection of *S. aureus* , *S. suis* and *P. multocida*, but after the treatment none of the bacteria could be isolated from the nasal swabs, indicating the recovery of animals due to treatment of plant extract. Considering the recovery days the plant extract @500 mg/mL/pig for 3 days was found to be the best dose for treatment of pigs having respiratory illness caused by the bacteria. The biotrim DS @500 mg/mL for 3 days was also useful for the treatment, but the use of the antibiotic may lead to the development of drug-resistant bacteria as the antibiotic sensitivity is seldom undertaken before the treatment. Since there is less chance for the emergence of resistant bacterial flora, the use of plant extract under field condition is considered to be most ideal for the treatment of respiratory diseases of pigs of bacterial etiology.

Identification and antibiotic susceptibility results of bacterial strains isolated from patients

Two Gram-positive bacteria, *S. aureus* and *S. pyogenes* as well as one GN bacteria, *K .pneumoniae* were isolated from the clinical samples of patients suffering from respiratory tract infection. The details of the bacteria isolated. *S. aureus* cultures were identified as MRSA and all the *Klebsiella* sp. was ESBL producing. In the antibiogram test, 20 antibiotics from 8 different groups were used against both GP and GN. All the bacteria were resistant to almost all the antibiotics used and they were effectively classified as MDR strains.

Antibacterial activity of plant extracts with the isolated bacteria from patients

Antibacterial activity of hydro-ethanolic fraction of *T. trilobatum* was monitored by the agar well diffusion method on separate lawn cultures of 3 bacterial isolates. The extract registered as the maximum diameter of zone of inhibition against MRSA (28 mm), *S. pyogenes* (29 mm) and ESBL *K. pneumoniae* (29 mm) (Table 9). Further, the MIC and MBC values of the extract were determined. The MIC value of 3.125 mg/mL and MBC value of 6.25 mg/mL was recorded against MRSA. Similarly, the MIC value of 1.56 mg/mL and MBC value of 3.125 mg/mL was recorded against *S. pyogenes*. However, for *K. pneumoniae* the MIC value was recorded as 1.56 mg/mL and the MBC value was recorded as 3.125 mg/mL.

CONCLUSION

The *In vivo* animal study using pig suffering from respiratory diseases revealed that the hydro-ethanolic extract of *T. trilobatum* very promising in the recovery of the animals compared to the standard antibiotics routinely used for the treatment. It is evident from the above findings that the nasal swabs taken from the experimental animals after giving treatment were free from the bacteria involved in the respiratory diseases. The animals were also found free from flu-like symptoms without any adverse reaction. This is well-supported as *In vitro* assessment of the antibacterial activity of the extract with the bacterial strains viz. *S. aureus* , *S. suis*, *P. multocida*, *E. coli*, *S. aureus* (ATCC) 33862 and *E. coli* (ATCC) 43888, isolated from the nasal swabs and tissues of the experimental animals. The extract was found to be very promising in killing the isolated bacteria compared to the clinically used antibiotics. More importantly, this extract was also found very effective against the MDR bacteria (both GP and GN) isolated from the clinical samples of patients suffering from respiratory diseases. Preliminary phytochemical analysis of the hydroethanolic extract confirmed the presence of three groups of compounds as organic acids, monosaccharides and polyols. The antibacterial activity of the plant may be attributed to the individual or combination of chemical groups. The findings of the present investigation offer scientific support to the ethnomedicinal use of *T. trilobatum* for the treatment and prevention of respiratory diseases in both pig and man.

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CONFLICTS OF INTERESTS

None

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Table 1: Bacteria isolated and identified from the nasal swabs and tissues samples of pigs suffering from respiratory tract infection.

Age group	No. of pigs examined	Type of samples	
		Nasal swab (86)	Tissue (38)
1-56 days (Suckling)	48 (AM) 20 (PM)	*S. aureus* (8)	S.suis (2) *E. coli* (6) *S. aureus* (5)
57-180 days (Weaners)	37 (AM) 10 (PM)	*P. multocida* (1)	*S. suis* (5) *P. multocida* (4) *E. coli* (4) *S. aureus* (8)
180days (Adults)	26 (AM) 4 (PM)	*P. multocida* (2)	*P. multocida* (1) *E. coli* (2) *S. aureus* (4) *S. aureus* MRSA (4)
Total	103	11	45

Table 2: Putative identification of the phytochemicals of the aqueous ethanol extract from rhizome of *Typhonium trilobatum*.

Peak#	Compounds present*	Retention Time of Silyl derivatives (min)	MW	Yield (%)
5	Lactic acid	11.786	90	0.47
6	L-valine	12.22	117	0.15
8	Benzoic acid	14.94	122	0.88
9	Glycerol	15.48	92	5.55
10	Butanedioic acid	16.00	118	0.28
11	Beta-hydroxypyruvic acid	17.18	104	0.22
12	Malic acid	18.57	134	1.95
14	D-Psicofuranose	22.27	180	18.82
15	α-Methyl galactoside	22.79	194	0.88
16	β-Methyl galactoside	23.14	194	0.72
17	1,3,4,5,6-pentahydroxy-D-Fructose	23.38	180	4.76
18	Methyl glucoside	23.64	194	1.10
19	Palmitic acid	24.95	256	29.14
20	9-Octadecenoic acid, methyl ester	25.48	282	4.57
21	Myo-Inositol	25.66	180	1.29
22	Oleic acid	26.71	282	13.84
23	Stearic acid	26.88	284	2.04

24	Hexadecanoic acid	27.36	568	2.36
25	9-Octadecenamide	28.01	281	0.72
26	13-Docosenoic acid	28.38	338	1.57
27	Glycidyloleate	28.96	338	7.09
28	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	29.13	625	0.97
29	1-Monopalmitin	29.85	330	0.63

*Bistrimethyl silyl trifluoroacetamideoctamethyltrisiloxane N, N'-methane tetraylbis [1,1,1 trimethyl-silanamine], Bis (trimethylsilyl) monomethyl phosphoric acid, Ribitolpentatrimethyl silyl derivatives are also detected in the gas chromatogram; but they are not listed in the table as they are added as silylating agents and ribitol is added as internal standard (vide experimentally).

Table 3. Compounds identified in methanolic extracts of *Typhonium trilobatum* using GC-MS analysis.

SN	RT	Compound Name	MW
i.	9.389	Chloro(Diethyl)Phosphine	124
ii.	11.295	Methyl 3-[(Trifluoroacetoxy)Methylthio]Prop-2-Enoate	244
iii.	12.61	Boronic Acid, Ethyl-, Bis(2-Mercaptoethyl Ester)	194
iv.	13.03	Methyl 6-Thia-Dodecanoate	232
v.	13.526	N-(1-Methoxycarbonyl-1-Methylethyl)-4-Methyl-2-Aza	203
vi.	14.256	4-Methylthiane, S-Oxide	132
vii.	15.801	Hexadecane, 1,16-Dichloro-	294
viii.	16.217	Trans-2-Methyl-4-N-Butylthiane, S, S-Dioxide	204
ix.	16.722	Methyl 9,10-Methylene-Hexadecanoate	284
x.	17.777	Phenol, 2,6-Bis(1,1-Dimethylethyl)-	206
xi.	18.167	DL-Proline, 5-Oxo-, Methyl Ester	143
xii.	18.893	Tetradecane, 1-Chloro-	232
xiii.	19.373	3-N-Hexylthiane, S, S-Dioxide	218
xiv.	19.948	Methyl 12-Methyl-Tridecanoate	242
xv.	20.944	Methyl 13-Methyltetradecanoate	256
xvi.	21.149	Methyl 14-Methylhexadecanoate	284
xvii.	21.674	Pentadecanoic Acid, Methyl Ester	256
xviii.	22.439	Methyl-D-Mannopyranoside	194
xix.	22.864	D-Glucopyranoside, Methyl	194
xx.	23.545	Methyl 11-Methyl-Dodecanoate	228
xxi.	23.69	Methyl 11-Hexadecenoate	268
xxii.	25.04	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	652
xxiii.	25.095	Methyl 15-Methylhexadecanoate	284
xxiv.	25.27	11-Bromoundecanoic Acid	264
xxv.	25.965	3-Pyrrolidin-2-Yl-Propionic Acid	143
xxvi.	26.991	Methyl 11,14-Octadecadienoate	294
xxvii.	27.301	Methyl 9-Cis,11-Trans-Octadecadienoate	294
xxviii.	27.491	Methyl 11,14,17-Eicosatrienoate	320
xxix.	27.851	Ethyl 9.Cis.,11.Trans.-Octadecadienoate	308
xxx.	28.251	Cyclopropanebutanoic Acid, 2-[[2-[[2-[(2-Pentylcyclop	374
xxxi.	28.367	Cis-13,16-Docasadienoic Acid	338

xxxii.	28.452	Ethyl 9,12-Hexadecadienoate	280
xxxiii.	28.712	4-Methyl-2,7-Dioxa-Tricyclo[4.4.0.0(3,8)]Decane	154
xxxiv.	28.952	1,10-Hexadecanediol	258
xxxv.	29.722	Methyl 8-Methyl-Nonanoate	186
xxxvi.	29.922	Methyl 9,12-Hexadecadienoate	266
xxxvii.	30.187	Butyl 9,12,15-Octadecatrienoate	334
xxviii.	30.242	Methyl 6-Cis,9-Cis,11-Trans-Octadecatrienoate	292
xxxix.	30.367	2-Nonyl-1-Ol, Diethyl Acetal	212
xl.	30.507	Butyl 6,9,12-Hexadecatrienoate	306
xli.	31.108	Methyl 11-Cyclopentylundecanoate	268
xlii.	31.703	Cis-13,16-Docasadienoic Acid	336
xliii.	32.458	Methyl 20-Methyl-Heneicosanoate	354
xliv.	32.683	Methyl 12,15-Octadecadienoate	294
xlv.	33.303	Methyl 2-Octylcyclopropene-1-Octanoate	308
xlvi.	34.554	2-Cyclohexylpiperidine	167
xlvii.	35.524	I-Propyl 7,10,13,16-Docosatetraenoate	374
xlviii.	36.33	Cyclohexanecarboxylic Acid, 4-Propyl-, 4-Cyanoph	271
xlix.	36.835	Geranylgeraniol	290

Table 4: Assignment of ^{1}H-NMR signals of acylglycerol groups presents in methanolic extracts of *Typhonium trilobatum* (Schott) corm, including chemical shift value, multiplicities and associated functional groups.

Signal	Chemical Shift (ppm)	Multiplicity	Functional group	Classification
A	0.743-0.872	triplet	$-CH_3$	Saturated, oleic acid and linoleic acyl groups
B	0.872-0.936	triplet	$-CH_3$	Unsaturated ω-3 acyl groups
C	1.116-1.346	triplet	-(CH2)n-	Acyl groups
D	1.461-1.625	m	$-OCO-CH_2-CH_2-$	Acyl groups except for DHA, EPA and ARA acyl groups
E	1.859-2.056	m	$-CH_2-CH=CH-$	Acyl groups except for -CH2- of DHA group in β position relative to the carbonyl groups
F	2.172-2.304	double triplet	$-OCO-CH_2-$	Acyl groups except for ω -6 acyl groups
G	2.653-2.725	triplet	$=HC-CH_2-CH=$	Unsaturated ω -3 acyl groups
H	2.726-2.781	triplet	$=HC-CH_2-CH=$	Unsaturated ω -3 acyl groups
I	3.448-3.506	singlet	-	-
J	3.656-3.687	singlet	$-OCH_3$	Methoxy group
K	4.014-4.282	double doublet, double doublet	$-CH_2OCOR$	Glycerol backbone groups
L	5.157-5.228	m	>CHOCOR	Glycerol backbone groups
m	5.228-5.367	m	-CH=CH-	Acyl chain olefinic functions

EPA, eicosapentaenoyl acyl groups; ARA, arachidonoyl groups.

Table 5: Results showing inhibition of growth of the bacteria by the plant extract at different concentrations.

Plant extract/ drug	Concentration (mg/ml)	Growth of the bacterial species in broth			
		Pasteurella multocida	*Escherichia coli*	*Streptococcus suis*	*Staphylococcus aureus*
Plant Extract	50	+	+	+	+
	100	+	+	+	+
	200	-	-	-	-
	300	-	-	-	-
Biotrim DS (Standard)	200	-	-	-	-
Broth with bacterial culture (Positive control)		+	+	+	+
Broth (Negative control)		-	-	-	-

+: Turbidity present; -: turbidity absent

Table 6. Antimicrobial activity of tuber extract of *Typhonium trilobatum* against isolated bacteria from pigs.

Concentration (mg/ml)	Diameter of zone of inhibition(mm)			
	S. aureus	*Strept. suis*	*P. multocida*	*E coli*
100mg/ml	-	-	-	-
200mg/ml	-	7mm	-	-
300mg/ml	10mm	14mm	7mm	-
500mg/ml	22mm	24mm	13mm	11mm

Table 7: Antibacterial activity of plant extract and its comparison with the standard antibiotics with bacteria isolated from pigs.

Bacteria	Antibiotic (mg/ml)								Plant extract (mg/ml)	
	Ceftriaxone		Tetracycline		Amikacin		Biotrim DS			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	2.0	2.0	1.5	4.5	3.5	5.5	1.0	1.0	5.3	21.0
S. suis	1.0	3.0	2.5	5.0	3.5	4.5	15.0	30.0	6.3	31.3
P. multocida	2.0	4.0	2.5	7.5	4.0	7.0	12.5	12.5	10.3	41.0
E. coli	3.0	3.0	5.0	10.0	3.5	3.5	6.5	6.5	14.5	58.0
S.aureus (ATCC)33862	1.0	1.0	0.5	1.0	2.5	2.5	3.0	3.0	4.3	21.3
E.coli (ATCC)43888	2.0	2.0	2.5	3.5	4.5	5.5	2.5	4.0	6.3	25.0

Table 8: Results showing recovery days of pigs after use of the plant extract and biotrim DS treated animals.

Group	Animal no.	Body wt (Kg)	Average Body wt (Kg)	Recovery (Days)	Antimicrobial used	Dose(mg)/ animal/day per os	Dose(mg)/ Kg Body wt
A	A1	15	17	3	Plant extract	500	29.41
	A2	16		3			
	A3	18		3			
	A4	17		3			
	A5	19		3			

B	B1	19	17	4	Biotrim DS tablet	500	29.41
	B2	15		3			
	B3	16		3			
	B4	20		4			
	B5	15		3			
C	CI	21	17	6	Plant extract	300	17.64
	C2	19		6			
	C3	14		5			
	C4	16		5			
	C5	15		5			
D	D1	15	17	7	Biotrim DS	300	17.64
	D2	16		7			
	D3	18		8			
	D4	17		8			
	D5	19		8			
E* (Control)	E1	17	17		No treatment		
	E2	20					
	E3	16					
	E4	13					
	E5	15					

Table 9: Antibacterial assay, MIC and MBC of *T. trilobatum*.

Isolated MDR bacterial strains	Zone of inhibition (in mm)		MIC (mg/ml)	MBC (mg/ml)
	Hydro-ethanolic extract	Linezolid/imipenem (30/10μg/ml)		
MRSA	28	26	3.125	6.25
S. pyogenes	29	30	1.56	3.125
K. pneumoniae	29	33	1.56	3.125

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration

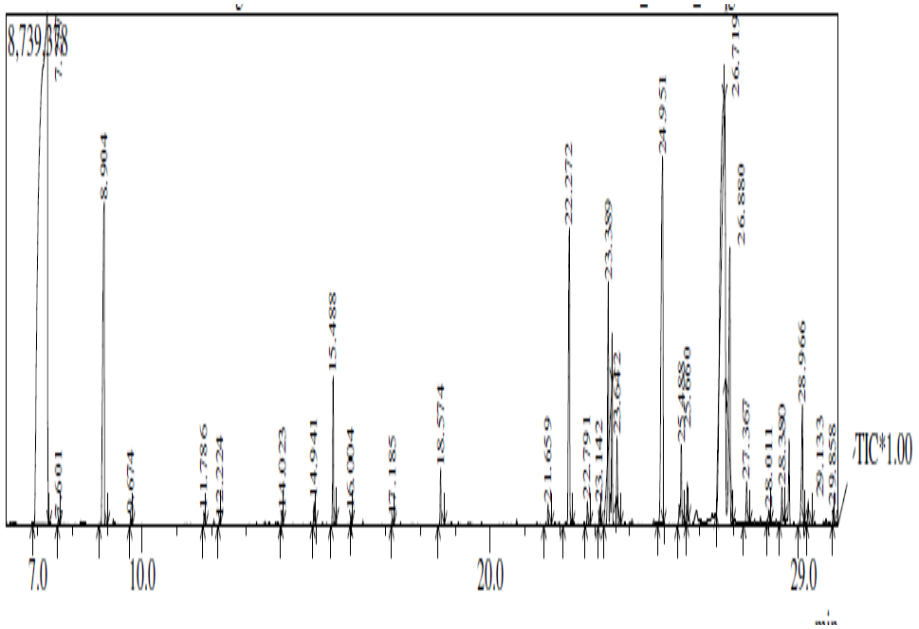

Fig.1: Chromatogram of the aqueous ethanolic extract showing the presence of different types of phytochemicals based on GC-MS analysis.

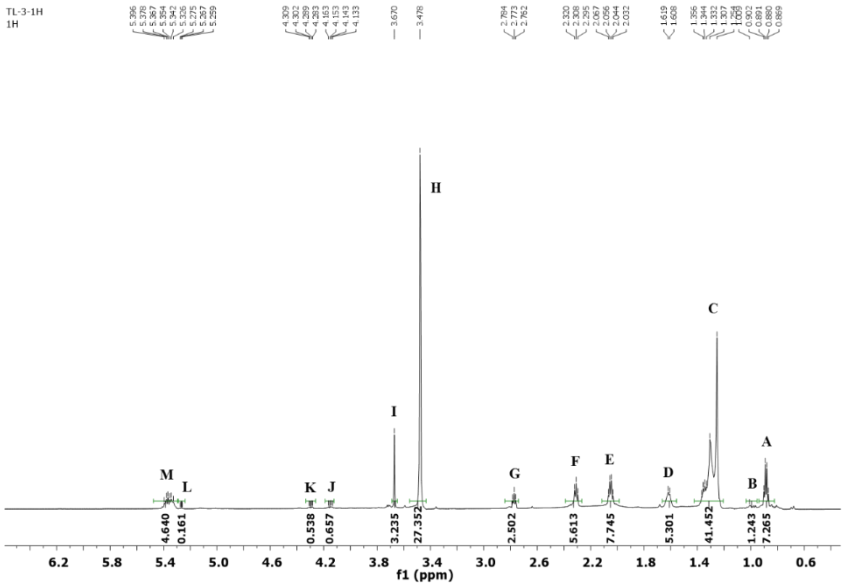

Fig.2: ^{1}H-NMR spectrum of *Typhonium trilobatum* methanolic extracts in $CDCl_3$. The alphabetic assignment represents the functional groups corresponding to the fatty acids and acyl groups present in methanolic extracts of *T. trilobatum* corm.

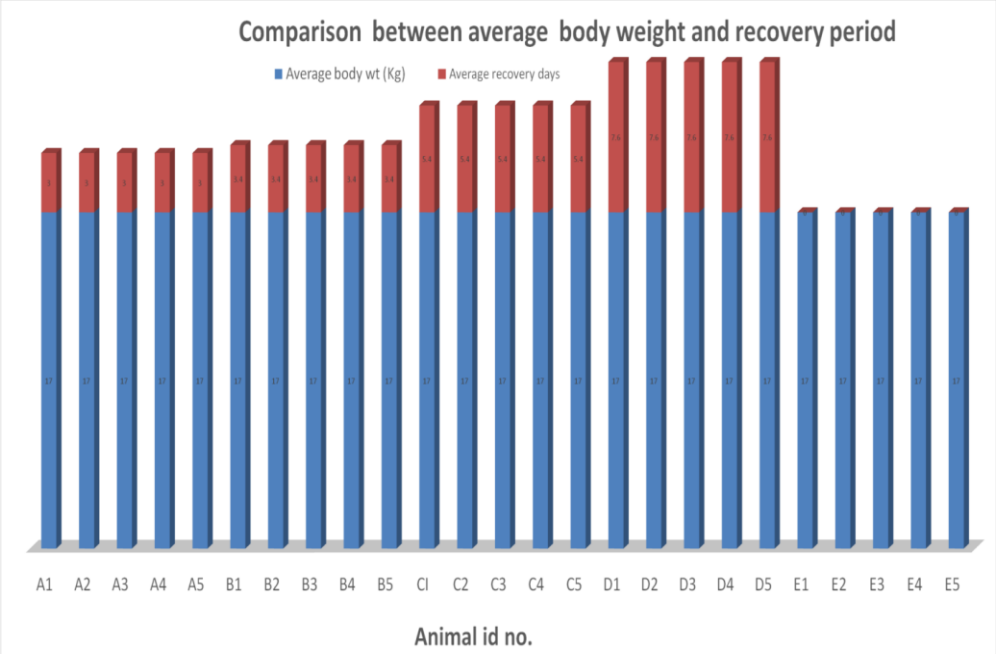

Fig.3: Results showing recovery days of pigs after use of the plant extract and biotrim DS. Animal ID No. A1-A5: Pigs received the plant extract @ 500 mg/ml/pig/day; Animal ID No.B1-B5: Pigs received biotrim DS @500 mg/ml/pig/day Animal ID No.C1-C5: Pigs received the plant extract @300 mg/ml/pig/day; Animal ID No.D1-D5: Pigs received the biotrim DS @300 mg/pig/day; Animal ID No. E1-E5: Control animals without any treatment