Efficacy of Metabolites from *Saccharum officinarum* Linn. Endophytic Fungi against some Uropathogenic Bacteria

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ABSTRACT

The in vitro antibacterial activities of endophytic fungal metabolites residing in stems of *Saccharum officinarum* Linn. was investigated on some bacterial species (*Escherichia coli, Staphylococcus aureus, Klebsiella* sp*. and Proteus* sp) isolated from clinical samples of urinary tract infections. Fresh pulp of *S. officinarium* was surface sterilized, crushed, and cultured on Potato Dextrose agar (PDA) containing antibiotics at room temperature for 7-10 days. Eleven (11) predominant pure colonies of *S. officinarium* endophytic fungi designated as *SoEnF1-11*, where then cultivated as single and mixed culture on potato dextrose broth shake flask for 15 days at room temperature. The mycelia were separated from the broth and extracted three times with ethyl acetate to obtain crude metabolite of each and mixed fungi culture. The antibacterial potential of the crude metabolites (*SoEnF 1 -11*) at concentration of 250 mg/ml were then determined against each of the test bacteria using the agar well diffusion techniques. Ciprofloxacin (30 µg/ml) was the reference standard drug used. The results revealed that the mixed crude fungal metabolites demonstrated the most promising antibacterial efficacy (14.3±1.6 - 22.0±0.5) against all the isolates when compared with the reference drug (22.1.0±0.1 -24.0±0.0). Significant antibacterial (8.0±1.8 - 18.4±0.1 mm) activity was also observed by each crude metabolite of *SoEnF1, 4, 5, 7, 8* and *10*, while *SoEnF2, 3, 6, 9* and *11* metabolites lacked significant biological activities. *S. aureus* was the most sensitive bacteria, while the least was *Proteus* sp. The MIC and MBC ranged between 15.6-125 mg/ml depending on the crude metabolite and test bacteria. The findings from this study suggest that some endophytic fungi present in stems of *S. officinarium* could be another potential source of novel antibacterial metabolites for production of future antibiotic agents against urogenital pathogens. Purification and molecular characterization of these endophytic fungi from *S. officinarum* visa-vice their essential bioactive metabolites for more detailed study is urgently needed.

Keywords: Endophytes, Sugar Cane, Antimicrobial agents, Agar well diffusion techniques

INTRODUCTION

The discovery and application of antibiotics years ago brought the hope of eradication of diverse infectious diseases of public health importance. As years rolled by, these infectious agents ones believed to have been controlled by antibiotics started developing new resistant forms to chemotherapeutic agents partly due to their extensive use and misuse (Levy and Marshall, 2004). There were calls for controlled use of antibiotics through the reduction of dosage used per regime of treatment or by regulating prescriptions in areas such as animal husbandry and aquaculture (Hernandez, 2005). While reduced use could lead to delayed resistance development, the emergence of resistant strains from an evolutionary viewpoint inevitable (Sibanda and Okoh, 2007). Hence, the need to continue the search for effective alternative and or complementary sources of antimicrobial compounds expecially from natural plant products. Currently, researchers have concentrated their studies on search for medicinal plants with bioactive compounds that can block or circumvent resistance mechanisms of the infectious agents (Sibanda and Okoh, 2007). This approach has been

yielding positive results because many plants with potential antimicrobial secondary bioactive compounds have been discovered (Pandey and Kumar, 2013).

Plants, apart from possessing phytochemical compounds, have also been discovered to habour several micro-oragainsms that reside in their inter- and intra-cellular tissues spaces and maintain a stable asymptomatic relationship with them. These groups of organisms are referred to as endophytes. Unlike mycorrhizal fungi that colonize plant roots and grow into the rhizosphere, endophytes reside entirely within plant tissues and may grow within roots, stems and/or leaves, emerging to sporulate at plant or host-tissue senescence (Stone et al., 2004). They have been found in every plant species examined to date and recognized as potential sources of novel natural products for exploitation in medicine, agriculture, and industry with more and more bioactive natural products being isolated from the endophytes (Arnold et al., 2000; Bacon and White, 2000). Since their discovery over 400 million years ago in many plant species, endophytic fungi have been found to play immense roles in the host plants such as synthesizing bioactive compounds that can be used by plants for defence against pathogens as well as other medically useful products (Arnold et al., 2000; Wang et al., 2002; Stinson et al., 2003; Owen and Hundley, 2004; Firakova et al., 2007; Desale and Bodhankar, 2013; Jain and Kumar, 2015). A recent survey revealed that the number of novel chemical structures produced by endophytic fungi was significantly higher than the soil fungi suggesting that these frequently overlooked endophytes might be the novel source of future antimicrobial agents (Khan et al., 2012). There is therefore the need to continually investigate various tropical and sub-tropical plants for their ability to host endophytic fungi having antimicrobial potential against infectious pathogens.

Saccharum officinarum (Linn.), commonly known as sugarcane belongs to the grass family (Poaceae). It widely grown mainly as source of sugar for human consumption and raw materials for alcoholic production (Ashade et al., 2014). Sugarcane has been used by humans as medicinal plants to manage diverse ailments. Both the roots and stems of sugarcane are used in ayurvedic medicine to treat skin and urinary tract infections as well as for bronchitis, heart conditions, and loss of milk production, cough and anemia (Pallavi et al., 2012). The peels have been reported to display a wide range of biological effects including immunological and thrombosis activity, chlorine release and anti-stress effects, anticancer and antitumor properties. The pharmacological potentials of this plant have been attributed to its major phyto-compounds such as anthocyanin, flavonoids and phenols which partially impart on colour of the juice (Pallavi et al., 2012; Ashade et al., 2014)

Urinary tract infections (UTIs) are the second most common type of infections accounting for morbidity and mortality in all human populations from neonates to the geriatric age group (Bag et al., 2013). Urinary tract infection is caused by pathogenic invasion of the urinary tract of the kidney, ureters, bladder, and the urethra with clinical manifestation depending of the region of the urinary tract involved, the pathogens and host immunity. The prevalence and incidence of urinary tract infection is higher in women than in men, which is likely the result of several clinical factors including anatomic differences, hormonal effects, and behaviour patterns (Akinjogunla et al., 2011). Some of the major bacteria commonly implicated in cases of UTis are mostly *Escherichia coli* and others bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella* sp. and *Proteus* spp. A high level of antibiotic resistance is very significant among the uropathogenic bacteria (Bag et al., 2013)

The study was therefore carried out to investigate the antibacterial efficacy of crude metabolites produced by endophytic fungal residing within the stem of *Saccharum officinarium* against some clinical isolates of urinary tract infection bacteria.

MATERIALS AND METHODS

Collection of Plant Sample

Healthy, matured stems of *Saccharum officinarum* that were free from insect, infections and mechanical damages were collected from farmlands located around Novena University, Amai Campus in July, 2014. The leaves and peels were trimmed off aseptically using sterile table knife, cut into smaller length and transported immediately to laboratory for analysis.

Source of Test bacteria

The bacterial species (*Escherichia coli, Staphyolococcus aureus, Klebsiella* sp*, and Proteus* sp.) used in this study were all of clinical origin and were sourced from clinical isolates from urine samples of urinary tract infected patients attending the University of Benin Teaching Hospital, Benin City, Nigeria. Their identities were further confirmed in the Laboratory using biochemical reactions as earlier described (Cheesebrough, 2010). Stock cultures were maintained on nutrient agar slant at 4^0C until needed.

Sterilization of plant sample Surface and fungal isolation

The collected stem samples were thoroughly washed with running tap water for 5-8 times and surface sterilized by immersing into 70% ethyl alcohol for 60 seconds, treated with 4% sodium hypochlorite solution for 3-4 min, and then again rinsed with 70% ethyl alcohol for 1 min. They were further rinsed with sterile water 5-8 times. A sterility test was thereafter conducted by plating aliquots of the sterile distilled water used in the final rinse onto the surface of potato dextrose agar (Hi media, India) plates supplemented with streptomycin (100 µg/ml) and the plates were examined for growth after incubation at 25°C for 3-5 days.

After sterility confirmation, the sterile stem pulp samples were reduced to tiny bits (about 1 x 1 cm^2) with the aid of sterile scalpel and asceptically inoculated onto sterile PDA pre-supplemented with streptomycin (100 µg/ml) to isolates the endophytic fungi. The inoculated plates were incubated at 25^0C and examined daily for 7-15 days for the development of fungal colonies growing out from the segments. On the basis of morphological characteristics, the different fungal mycelia growing out of the sample plates were selected, sub-cultured and the pure endophytic fungi maintained in PDA plates (Jain and Kumar, 2015).

Fermentation and Extraction of Endophytic Fungal Metabolites

The method described by Tayung et al. (2010) was followed. Potato dextrose broth was prepared according to specification. Then, 200 ml was measured and dispersed into 500 ml flasks each for the cultivation of the fungal isolates. The medium are were autoclaved and later inoculated with four discs plugs (6mm in diameter) of fresh mycelia of the different endophytic fungi cultures grown on PDA for 7 days. All the flasks were plugged with cotton wools and incubated at room temperature 25^0C for 14 days with periodic manual shaking. The fermentation broth of each endophytes was filtered through cheesecloth to separate the mycelia biomass. The filtrate was then extracted exhaustively three times ethyl acetate (1:1) in a separating funnel by vigorous mixing for 30-40 min. The organic phase (Ethyl acetate) containing the fungal metabolites is separated and concentrated using rotary evaporator to obtain the crude metabolite extract for antimicrobial assay.

Assay of crude metabolite extract of endophytic fungal cultures

The antibacterial activity of the extract was determined using agar well diffusion method as described by Hamid and Aiyelaagbe (2011). An overnight culture of each bacterium was prepared by taking two loopful from the stock cultures into sterile 5 ml nutrient broth and incubated for 18-24 hr at 37^oC. From the overnight cultures, 0.1ml of each organism was aseptically inoculated into 9.9ml of sterile distilled

water to obtained 10^{-2} inoculum concentration of the organism. From the diluted organism (10^{-2}), 0.2ml was aseptically inoculated prepared sterile molten Mueller Hinton Agar (Hi-media India) Petri dishes, mixed properly and allowed to solidify. With a sterile cork-borer (8mm diameter) four (4) wells were made on each solidified plate and the bottom of each hole was covered with molten agar. 100µl of crude endophytic fungal metabolite extract dilution (250 mg/ml) was added into each well on different plates. 100µl of Ciprofloxacin (30µg/ml) was used as positive control while organism seeded-plates without antibacterial agents were used as negative control. The Petri dishes were left on the bench for about 2hrs to allow the extract diffuse properly into the agar. They were then incubated at 37^{o}C for 24 h and zone of inhibition were observed and measured using transparent ruler in millimeters. The procedure was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined only for the crude metabolite extracts and on the isolates for which it showed inhibitory activity. Broth dilution technique of Cheesbrough, (2010). was employed to determine the MIC of the potent extracts. Doubling dilution of the potent extracts were prepared using peptone water (prepared) to obtain a series of dilution containing 250, 125, 62.5, 31.25, 15.63, 7.81mg/ml of fungal metabolites. Standardized inoculum of the test organisms (exactly 0.02ml) compared with the MacFarland turbidity standard was inoculated into each of the5 set of test tubes containing 2ml of the mixture. Tubes containing extract-free broth were used as control, and all were incubated together at 37^{0}C for 24 hours. MIC was read as the least concentration that showed no growth, using turbidity as a measure (Ezeadila et al., 2015).

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by collecting 0.2 ml of broth culture from the tubes used for the MIC determination (starting from the MIC point backwards) and subculturing onto fresh extract-free and drug-free solid agar plates. The plates were incubated at 37 ^{0}C for 24 hours. The least concentration that didn't show any growth after 24 hours of incubation was regarded as the MBC (Ezeadila et al., 2015).

Statistical Analysis

Calculation of means and standard deviations, using Microsoft Excel office 2007 version. Test of significance were performed using SPSS 16.0 version for Windows program (SPSS, Inc.)

RESULTS AND DISCUSSION

Endphytic fungi are famous for their metabolic potentials in producing diverse group of bioactive molecules to protect plants against plant and animal pathogens (Santos et al., 2015). The search for plants with biologically active endophytic microorganisms continues unabated. This is important in the face of incessant reports on the rising trends of emerging, re-emerging and multiple drug resistant pathogens to currently available arsenal of chemotherapeutic agents.

In this study, the endophytic fungi present in *S. officinarium* were screen for their abilities to produce bioactive molecules with chemotherapeutic potentials against some common clinical isolates of urinary tract infections (UTIs). The results of this study revealed that most of these endophytic fungi tested possess marked antibacterial metabolites. Table 1 shows the antibacterial activities of crude metabolite extracts of the eleven predominant *S. officinarium* endophytic fungal isolates (*SoEnF).* From the results, it was observed that *SoEnF1, 4, 5, 7, 8* and *10* possess antibacterial bioactive molecules, while *SoEnF2, 3, 6, 9* and *11* lacked same, based on the fact that there were no measurable zones of

inhibition. Among those with antibacterial metabolites, the highest mean zone of inhibition was recorded by *SoEnF4* (18.4 ± 1.1 mm) against *S. aureus*¸ while the least was seen in *SoEnF1* (8.0 ± 1.8 mm) against *Proteus* sp. The activities of the best endophytic fungi (*SoEnF4)* was however not significantly different (P<0.05) from those of *SoEnF5* and *SoEnF8* against *E. coli, S. aureus, Klebsiella* sp, and *Proteus* sp. The observed antibacterial activities further confirmed that these endophytes produced antibacterial metabolites.

Although, all the extracts demonstrated significant activities against both the gram positive (*S. aureus*) and gram negative (*E. coli, Klebsiella* sp. and *Proteus* sp.) test bacteria, it appears that the gram positive bacterium (*S. aureus*) was generally the most sensitive. The possible suggestion to this finding may not be unconnected with the differences in the anatomical structure and physiology of their cell walls as reported earlier ((Joshi et al., 2009; Katsa et al., 2014). The inhibition of both group of test bacteria also indicates that the crude metabolites extracts possibly possess a broad spectrum antibacterial activities. This result is in agreement with the findings of (Santos et al., 2015), who reported that *Nigrospora sphaerica,* an endophytic fungi residing in the leaves of *Indigofera suffruticosa* possess broad spectrum activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The lack of *in vitro* biological activities by some of the endophytic fungi may not be enough to conclude that they lack antibacterial compounds. According to Strobel et al. (2004), the amount and kind of bioactive compounds produced by an endophytic fungus are controlled by the incubation temperature, medium composition and degree of aeration. Hence, temperature, agitation and medium composition as well as its pH might have unfavoured role for optimal secretion of their bioactive metabolites. Moreover, the bacteria might be resistant to the metabolites produced by these endophytes special mechanisms as reported earlier (Sibanda and Okoh, 2007). Findings on lack of antibacterial activities of pathogens to most endophytic fungi was recently reported (Ratnaweera et al., 2015).

Our study further revealed that the mixture of all the metabolites of each endophytic fungus yielded the most promising antibacterial activities against the test pathogens. Such outstanding result could possibly reflect the availability of a higher amount of the metabolites which directly translated into relatively higher effect. This finding is worth noting because the inhibition values obtained against the test bacteria (*E. coli, S. aureus* and *Klebsiella* sp.) were generally not statistically different (P=0.05) from those obtained with Ciprofloxacin (30µg/ml), the reference standard positive control drug. It therefore suggests that these endophyic fungal metabolites, when purified could be a major promising antimicrobial agent especially against these urinary tract pathogens which had been documented to exhibit a high level of multiple-drug resistance to common antibiotics (Bag et al., 2013). This result further buttress the fact that the enthno-medicinal claim of using stems of *S. officinarum* in treating urinary tract infections among others might be partly due to some of these metabolites produced by the inherent endophytic fungi.

Table 1: Antibacterial Activity of Endophytic fungal metabolites on test bacteria

	Mean Diameter Zones of Inhibition (mm ± SD)			
Crude Extract (250mg/ml)	*E. coli*	*S. aureus*	*Klebsiella* sp	*Proteus* sp
SoEnF1	10.7 ± 2.2	12.8 ± 0.5	11.2 ± 0.4	08.0 ±1.8
SoEnF2	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
SoEnF3	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
SoEnF4	09.2 ± 3.1	18.4 ± 1.1*	10.3 ± 1.8	14.9 ± 0.6*
SoEnF5	18.1 ± 1.4*	15.9 ± 2.4*	13.6 ± 1.3*	16.5 ± 1.7*
SoEnF6	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
SoEnF7	12.6 ± 2.1	12.9 ± 0.6	09.8 ± 0.8	10.7 ± 0.3
SoEnF8	15.5 ± 2.4*	15.7 ± 1.9*	12.7 ± 1.7	15.9 ± 0.4*
SoEnF9	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
SoEnF10	09.9 ± 0.2	10.6 ± 1.5	10.2 ± 1.6	09.4 ± 0.9
SoEnF11	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
Mixed (*SoEnFm1-11*)	22.0 ± 0.5**	19.6 ± 2.1**	16.9 ± 1.9**	14.3 ± 1.6*
Ciprofloxacin (30µg/ml)	23.0 ± 0.1**	24.0 ± 1.0**	22.1 ± 0.1**	22.5± 0.0**

SoEnF = S. officinarium Endophytic fungal isolates*; *P<0.05, **P<0.05*

Table 2. Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of crude endophytic fungal metabolites

	MIC/MBC (mg/ml)			
Crude Extract	*E. coli*	*S. aureus*	*Klebsiella sp*	*Proteus sp*
SoEnF1	125/125	62.5/125	62.5/125	125/125
SoEnF4	62.5/125	31.25/62.5	125/125	62.5/62.5
SoEnF5	62.5/62.5	31.25/62.5	62.5/62.5	62.5/125
SoEnF7	125/125	62.5/125	62.5/125	125/125
SoEnF8	62.5/125	62.5/62.5	125/125	62.5/125
SoEnF10	62.5/125	125/125	125/125	62.5/125
Mixed (*SoEnF1-11*)	15.63/31.25	15.63/15.63	31.25/62.5	31.25/62.5

The results of the MIC and the MBC of individual and mixed endophytic fungal metabolites are presented shown in Table 2. MIC and MBC tests are employed frequently to assess some diverse non-antibiotic- and antimicrobial agents such as antibiotics, antiseptics, disinfectants and chemotherapeutic agents (Croshaw, 1983; Acheampong et al., 1988). Antimicrobial agents with low activity against an organism usually gives a high MIC and MBC values, while those that are highly effective give low MIC and MBC values (El-Mahmood, 2009). Our results showed that the MIC of the endophytic fungal metabolites ranged from 31.25 - 125 mg/ml, while the MBC ranged from 62.5 - 125 mg/ml (Table 2). The mixed extract gave relatively lower MIC/MCB values of 15.63 and 31.25 mg/ml. The low MIC and MBC values recorded by the combined crude metabolite extract reaffirmed our earlier assertion that bioactive molecules, when combined, could demonstrate a multiplier effect. Also, the low values recoded by the metabolites of *SoEnF4* and *5* against *S. aureus* further revealed that they were the most susceptible test bacteria. In general, the relatively low values of MIC/MBC, and in most cases similar MIC/MBC values (Table 2) recorded by the endophytic fungal metabolites against the test isolates implies a high activity of the extract at low concentrations. High activity of antimicrobial agent at low

concentration, in relation to the standard reference drug is very essential for chemotherapeutic purposes because of their toxicity to the patient's system (Ogu et al., 2012). It thus, confirms the potential efficacies of the crude metabolites. This research was conducted on crude extract; it is believed that if the metabolites are further purified, stronger inhibitory results will be achieved.

CONCLUSION

This study has revealed that the stem of *S. officinarum* harbours diverse endophytic fungi which are capable of synthesising bioactive metabolites with broad spectrum antibacterial activities. Although, the individual biological activities of six of the endophytic fungal metabolites (*SoEnF*1, *SoEnF*4, *SoEnF*5, *SoEnF*7, *SoEnF*8 and *SoEnF*10) were significant, the most promising activity was achieved when they were mixed together. Hence, the cultivation of these endophytic fungi under appropriate growth medium and environmental conditions could be another potential source of generating novel antibacterial metabolites for production of future antibiotic agents against urogenital pathogens. However, purification and molecular characterization of these endophytic fungi from *S. officinarum* visa-vice their essential bioactive metabolites for more detailed study is urgently needed.

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REFERENCES

1. Acheampong, Y. B., El-Mahmood, A. M. & Olurinola, P. F. 1988. Antibacterial properties of the liquid antiseptic TCP. Indian J. Pharm. Sci. 3, pp. 183-186

2. Akinjogunla, O. J., Etok, C. A. & Oshoma. C. E. 2011. Preliminary phytochemistry and in vitro antibacterial efficacy of Hydro-Ethanolic leaf extracts of *Psidium guajava* on common urinary tract Bacterial Pathogens. Biores. Bulletin. 1, pp. 065-072

3. Arnold, A. E., Maynard, Z., Gilbert, G. S. Coley, P. D. & Kursar, T. A. 2000. Are tropicalfungal endophytes hyper-diverse? Ecol. Lett. 3, pp. 267-274.

4. Ashade, O. O., Abubakar, R. O., Nguka, O. O., Yakubu, A. O., Oyesanya, O., Ofoegbu, C. C., Bello, O. N. & Osuntade, B. A. 2014. Impact of Sugar cane peel (*Saccharum officinarum*) Extract on the Blood Status and Gonadal Integrity of Wistar Albino Rat. Int. J. Res. Pharm. Chem. 3(3), pp. 646-655.

5. Bacon, C. W. & White, J. F. J. 2000. "Microbial Endophytes" Marcel-Dekker Inc., New York, Basel. p. 487.

6. Bag, A., Bhattacharyya, S. K., Pal, N. K. & Chattapadhyay, R. R. 2013. Antibacterial potential of hydroalcoholic extracts of Triphala components against multidrug resistant uropathogenic bacteria- a preliminary report. Indian J. Exp. Biol. 51, pp. 709-714.

7. Cheesbrough M, 2010. District Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, Canbridge, UK, pp. 40-197.

8. Croshaw, B. 1983. Evaluation of non-antibiotic antimicrobial agents. In: Pharmaceutical Microbiology, 3rd edn., Hugo WB and Russell AD editors. Blackwell, Oxford, pp. 237-257

9. Danielsen, S. & Jensen, D. F. 1999. Fungal Endophytes from Stalks of Tropical Maize and Grasses: Isolation, Identification, and Screening for Antagonism against *Fusarium verticillioides* in Maize Stalks. Biocontrol. Sci. Technol. 4(1), pp. 545-553.

10. Desale, M. G. & Bodhankar, M. G. 2013. Antimicrobial Activity of Endophytic Fungi Isolated from *Vitex negundo* Linn. Int. J. Curr. Microbiol. App. Sci. 2(12), pp. 389-395.

11. El-Mahmood A. M. 2009. Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniea and Pseudomonas aeruginosa.* J. Med. Plants Res. 3(4), pp. 179-185.

12. Ezeadila, J. O., Nwande, M. O., Ogu, G. I. & Aneke, F. A. 2015. Antibacterial activity of ethyl acetate and n-hexane extracts of *Vernonia amygdalina* and *Moringa oleifera* leaves on *Staphylococcus aureus* and *Escherichia coli* isolated from urine samples. Adv. Appl. Sci. Res. 6 (11), pp. 23-28.

13. Firakova, S., Sturdikova, M. & Muckova, M. 2007. Bioactive secondary metabolites produced by micro-organism associated with plants. Biologia. 62, pp. 251-257.

14. Hamid, A. A. & Aiyelaagbe, O. O. 2011. Preliminary Phytochemical, Antibacterial and Antifungal Properties of *Alafia barteri* Stem Grown in Nigeria. *European J. Med. Plants.* 1(2), pp. 26-32,

15. Hernandez, S. P. 2005. Responsible use of antibiotics in aquaculture. FAO Fisheries Technical paper. p. 469.

16. Jain, P. & Kumar, T. 2015. Isolation of endophytic fungi from leaves of A*zadirachta indica* and preliminary screening for antimicrobial activity. World J. Pharm. Res. 4(1), pp. 1882-1891.

17. Joshi, B., Lekhak, S. & Sharma, A. 2009. Antibacterial property of different medicinal plants: ocimum sanctum, *Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*. Kathmandu Univ. J. Sci. Eng. Technol. 5(1), pp. 143-150.

18. Katsa, M., Gyar, S. D. & Reuben, C. R. 2014. *In Vitro* Antibacterial Activity of *Allium roseum* (Wild Allium or Rosy Garlic) against Some Clinical Isolates. J. Microbiol. Res. Rev. 2(4), pp. 30-33

19. Khan, R., Shahzad, S., houdhary, M. I. C., Khan, S. A. & Ahmad, A. 2007. Biodiversity of the endophytic fungi isolated from *Calotropis procera*. Pak. J. Bot. 39(6), pp. 2233-2239.

20. Levy, S. B. & Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10, pp. 122-129.

21. Ogu, G. I., Tanimowo, W. O, Nwachukwu, P. U. & Igere, B. E. 2012. Antimicrobial and Phytochemical Evaluation of the Leaf, Stem Bark and Root Extracts of *Cyathula prostrata L. Blume* against some Human Pathogens. J. Intercult. Ethnopharmacol. 1(1), pp. 35-43.

22. Owen, N. L. & Hundley, N. 2004. Endophytes the chemical synthesizers inside plants. Sci. Prog. 87, pp. 79-99.

23. Pallavi, P., Elakkiya, S., Tennety, S. S. R. & Devi P. S. 2012. Anthocyanin analysis and its Anticancer Property from Sugarcane (*Saccharum officinarum L*) Peel. Int. J. Res. Pharm. Chem. 2(2), pp. 338-345.

24. Pandey, A. K. & Kumar, S. 2013. Perspective on plant products as antimicrobial agents: a review. Pharmacologia. pp. 469-480.

25. Ratnaweera, P. B., Dilip de Silva, E., Williams, D. E. & Andersen, R. J. (2015). Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic *Fusarium* sp. BMC Complem. Altern. Med. 15, 220, pp. 1-7

26. Santos, I. P., Silva, L. C. N., Silva, M. V., Araújo, J. M., Cavalcanti, M. S. & Lima, V. L. M. 2015. Antibacterial activity of endophytic fungi from leaves of *Indigofera suffruticosa* Miller (Fabaceae). Front. Microbiol. 6, 350.

27. Sibanda, T. & Okoh, A. I. 2007. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr. J. Biotechnol. 6 (25), pp. 2886-2896.

28. Stinson, M. Ezra, D. Hess, W. M., Sears, J. and Strobel, G. 2003. An endophytic *Gliocladium* sp. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. Plant Sci. 165, pp. 913-922.

29. Stone, J. K., Polishook, J. D. & White, J. R. J. 2004. Endophytic fungi. In: Mueller G, Bills G F, Foster M S, eds. *Biodiversity of fungi: inventory and monitoring methods*. Burlington, MA, USA. Pp. 241–270.

30. Strobel, G., Daisy, B., Castillo, U., & Harper, J. 2004. Natural products from endophytic micro-organisms. J. Nat. Prod. 67, pp. 257-268.

31. Tayung, K., Barik, B. P., Jha, D. K & Deka, D. C. 2010. Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Fusarium solani* isolated from bark of Himalayanyew. Mycosphere. 2(3), pp. 203–213, 2010.

32. Wang, J., Huang, Y., Fang, M., Zhang, Y., Zheng, Z., Zhao, Y., Su, W. & Brefeldin, A. 2002. A cytotoxin produced by *Paecilomyces* sp. and *Aspergillus clavatus* isolated from *Taxus mairei* and *Torreya grandis*. FEMS Immunol. Med. Microbiol. 34, pp. 51-57.