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A PROMISING ALTERNATIVE FOR TREATMENT OF BACTERIAL INFECTIONS BY *SANSEVIERIA CYLINDRICA* BOJER EX HOOK LEAF EXTRACT

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The aim of this study was to assess the *in vitro* antibacterial activity of ethanolic extract prepared from *Sansevieria cylindrica* Bojer ex Hook leaves against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* strains, clinically important bacteria, which are indicator organisms commonly used in programs to monitor antibiotic resistance. For this study, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* NCTC 12493, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27583 were used. The disc diffusion assay (Kirby–Bauer method) was used to screen for antibacterial activity of leaf extract. The results of antibacterial activity clearly showed that the extract has shown antibacterial activity against the entire tested organisms. The extract has shown better activity against *S. aureus* and *P. aeruginosa* strains compared to the *E. coli* strains. The diameters of inhibition zones were (22.5 ±1.24) mm, (20.5 ±1.3) mm, and (16.4 ±0.95) mm for *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, and *S. aureus* NCTC 12493, respectively. The extract has shown less antimicrobial activities against *P. aeruginosa*. Finally, the ethanolic extract exhibited mild antibacterial activity against *E. coli*. Further chemical analysis of the aforementioned plant extract should be performed to determinate their chemical composition and identify the exact secondary metabolites responsible for the antimicrobial activity. In addition, the extract should be subjected to pharmacological evaluations with the aim of assessing its efficacy and toxicity, interactions and contraindications.

Keywords: *Sansevieria cylindrica* Bojer ex Hook, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, leaves, ethanolic extract, antimicrobial activity, agar disk diffusion assay

Introduction

The emergence of human pathogenic microorganisms that are resistant to major classes of antibiotics has increased in recent years, due to the indiscriminate use of commercially available antimicrobial agents. It was suggested that resistance mechanisms probably have evolved from genes present in organisms that produce antibiotics (Hawkey, 1998). The presence of antibiotic-resistant strains among food derived microorganisms suggests that

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it may play a much more important role in transferring the antibiotic resistance encoding genes than previously thought (Chajęcka-Wierzchowska et al., 2016; Bielikova et al., 2017). Considering, that antibiotics are used for prophylactic purposes in livestock and poultry production, in vegetable and fruit cultivation and as well as in beekeeping (Ding and He, 2010), it was suggested that the meat of farm animals, fruit, vegetables, and water may be a source of resistant strains (Chajęcka-Wierzchowska et al., 2017).

Sansevieria Thunb., a genus with diverse ethnobotanical uses in its geographical distribution range, has occupied an important place among plant genera applied for treatment of a broad spectrum of diseases and disorders (Khalumba and Mbugua, 2005; Staples and Herbst, 2005; Takawira-Nyenya et al., 2014). Comprehensive information concerning ethnobotanical uses of various *Sansevieria* species in Kenya was presented and critically evaluated by Takawira-Nyenya and coauthors (2014). These authors reported that ethnobotanical data on various *Sansevieria* species have been well documented in various locations in East Africa. For example, Bally (1937) reported that *S. kirkii* Baker roots are used for the treatment of foot sores (cited by Takawira-Nyenya et al., 2014). As previously described, *S. trifasciata* Prain has been used for the treatment of inflammatory ailments and snakebite (Morton, 1981). Moreover, *S. trifasciata* are used in folk medicine for treating bronchitis, asthma, food poisoning, toxemia, cough, snake bite, insect bite etc. (Seth, 2005). The *S. trifasciata* extracts possess mild analgesic properties and elicit analgesic-, anti-inflammatory and antipyretic activity in mice (Anbu et al., 2009).

The other documented folk medicinal uses of *Sansevieria* species include treatment for abdominal pains, diarrhea, and hemorrhoids (Andhare et al., 2012). In traditional health care practice, the leaves of *S. liberica* Gérôme and Labroy are used as painkillers, and in the treatment of smallpox, chicken pox, measles and most venereal diseases (Chigozie and Chidinma, 2013). It was reported that preparations of *S. liberica* plant are used in the treatment of ear and eye infections, inflammation (leaf juice); tooth-ache (fruit juice together with fluid from snails); fever, headache, and cold (fume from burning leaves inhaled); cough, pain, inflammation, infections, convulsion, diarrhoea, and as stimulating tonic (root decoction) (Watt and Breyer-Brandwijk, 1962). The hydroethanolic extract of *S. liberica* possesses also significant anticancer activity (Akindele et al., 2015). The pressed juice of the *S. liberica* leaves is dropped in the eyes and ears for the treatment of infections and inflammations (Chigozie and Chidinma, 2013). Additionally, the *S. liberica* roots are used for the treatment of convulsion, epilepsy, paralysis, malnutrition, pulmonary troubles, vermifuges, cough and debility (Ikewuchi et al., 2011). In Nigeria, the leaves and roots of *S. liberica* are used in traditional health-care practice for the treatment of asthma, abdominal pains, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, diabetes mellitus, menorrhagia, piles, sexual weakness, wounds of the foot, and alleviating the effects of snake bites (Chigozie and Chidinma, 2013). The anti-anemic and sedative and anticonvulsant activities of the *S. liberica* leaves and roots have been reported (Ikewuchi et al., 2010; Adeyemi et al., 2007).

The roots and rhizomes of *S. roxburghiana* Schult. & Schult. f. are used in the traditional medicine as the remedies for diabetes, inflammation, pains, fever, asthma, wound, hypertension, oxidative stress and rheumatism (Dhiman, 2006; Pulliah, 2006; Haldar et al., 2010). *S. roxburghiana* could offer an overall protective effect through attenuating hyperglycemia and arresting inflammation in type 2 diabetes and its associated cardiomyopathy (Bhattacharjee et al., 2016). *S. senegambica* Baker is used in traditional health practice in southern Nigeria for treating bronchitis, inflammation coughs, boils and hypertension. It is also used in arresting the effects of snake bites, as well as in compounding solutions used as hair tonics. A dose-dependent hypocholesterolemic effect of the extract suggesting a likely protective role of the extract against dyslipidemia and the development of cardiovascular diseases was also revealed (Ikewuchi, 2012). African *S. ehrenbergii* were shown to possess anticancer activity against the P388 lymphocytic leukemia cell line and a panel of human cancer cell lines. Sansevistatin 2 and other saponins isolated from the same source, exhibited antifungal activity against *Candida albicans* and *Cryptococcus neoformans* (Pettit et al., 2005). From the leaves of *S. cylindrica*l Bojer ex Hook, a new steroidal saponin was isolated and showed inhibition of the capillary permeability activity (Da Silva Antunes et al., 2003). Watt and Breyer-Brandwijk (1962) listed the use of *S. hyacinthoides* (L.) Druce in the treatment of a toothache and earache and the use of the rhizome decoction of *S. kirkii* as a purgative both reported from East Africa. Yet, Kiringe (2006) reported on the use of *S. volkensii* Gürke for the treatment of sexually transmitted diseases such as gonorrhea. In Kenya, Owuor and Kisangau (2006) included the use of *S. parva* N.E.Br. leaf sap and *S. kirkii* Baker extracts for treatment of snake bite wounds. Nevertheless, in spite of these data, Takawira-Nyenya et al. (2014) reported that the documentation of ethnobotanical uses of genus *Sansevieria* is incomplete.

Sansevieria species also showed antimicrobial activity (Onah et al., 1994; Aliero et al., 2008; Philip Deepa et al., 2011; Sheela et al., 2012). In our previous study, we have evaluated the antibacterial capacity of ten species of *Sansevieria* genus against *Staphylococcus aureus* in order to validate scientifically the inhibitory activity for microbial growth attributed by their popular use and to propose new sources of antimicrobial agents (Buyun et al., 2016). The selected bacterial strain *S. aureus* is widespread and causes serious problems due to their pathogenicities and high levels of drug resistance. This has caused many clinical problems in the treatment of infectious diseases because the commercially available antibiotics commonly used are sometimes associated with adverse effects such as hypersensitivity, allergic reaction, and immunosuppression in the host. Thus, the search for the discovery of new antimicrobial agents is an urgent need. The results proved that the inhibition zones ranged between 16 and 34 mm. *S. fischeri* and *S. francisii* extracts were particularly active against tested strain (diameters of inhibition zones were 34 mm). This was followed by the activities of *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, *S. metallica* leaf extracts (diameters of inhibition zones ranged from 25 to 31 mm). The ethanolic extracts of *S. canaliculata* and *S. trifasciata* showed less antimicrobial activities (16 to 16.5 mm). The results proved that the ethanolic extracts of *S. fischeri*, *S. francisii*, *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, *S. metallica* exhibited a favorable antibacterial activity against *S. aureus*. By the agar diffusion method, the ethanolic extracts of *S. fischeri*, *S. francisii*, *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, and *S. metallica* leaves showed anti-*S. aureus* activity, evidencing that ethanol is an efficient organic solvent to be used for the extraction of bioactive plant materials (Buyun et al., 2016). As previously mentioned, our results also revealed that the ethanolic extracts obtained from leaves of *S. kirkii*, *S. arborescens*, *S. roxburghiana*, *S. francisii*, *S. forskaliana*, *S. cylindrica*, *S. trifasciata*, *S. canaliculata*, *S. caulescens*, *S. metallica*, *S. aethiopica* possess antibacterial potency against *Escherichia coli* isolates and may be used as natural antiseptics and antimicrobial agents in medicine (Tkachenko et al., 2017).

Consequently, the results of the present study confirm the importance of the studied plants of *Sansevieria* species as a source of bioactive compounds for the treatment of infectious diseases. Therefore, the current study was designed to test the efficacy of ethanolic extract prepared from *S. cylindrica* Bojer ex Hook leaves against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* strains, clinically important bacteria, which are indicator organisms commonly used in various projects in order to monitor antibiotic resistance (Boss et al., 2016).

Materials and methodology

Collection of plant material and preparation of plant extract

Totally expanded leaves of *Sansevieria cylindrica* plants were sampled for study (Figure 1A). *S. cylindrica* is a most distinct stemless succulent plant that grows fan-shaped, with stiff leaves growing from a basal rosette. It forms in time a colony of solid cylindrical leaves (Figure 1B). The species is interesting in having cylindrical instead of strap-shaped leaves. It spreads by rhizomes – roots that travel under the soil surface and develop offshoots some distance from the original plant. Rosette is formed a few leaves distichous rosettes with 3–4 leaves (or more) from underground rhizomes. Leaves are round in cross-section, leathery, rigid, erect to arching, channelled only at the base, dark-green with thin dark green vertical stripes and horizontal grey-green bands about (0.4)1–1,5(-2) m in height and about 2–2,5(-4) cm thick. The 2.5–4 cm flowers are tubular, delicate greenish-white tinged with pink and lightly fragrant. Blooming season: It blooms once a year in Winter to Spring (or summer too) (http://www.llifle.com).

Freshly collected leaves were washed, weighted, crushed, and homogenized in 96% ethanol (in proportion 1 : 19) at room temperature. The extracts were then filtered and investigated for their antimicrobial activity. All extracts were stored at 4 °C until use.

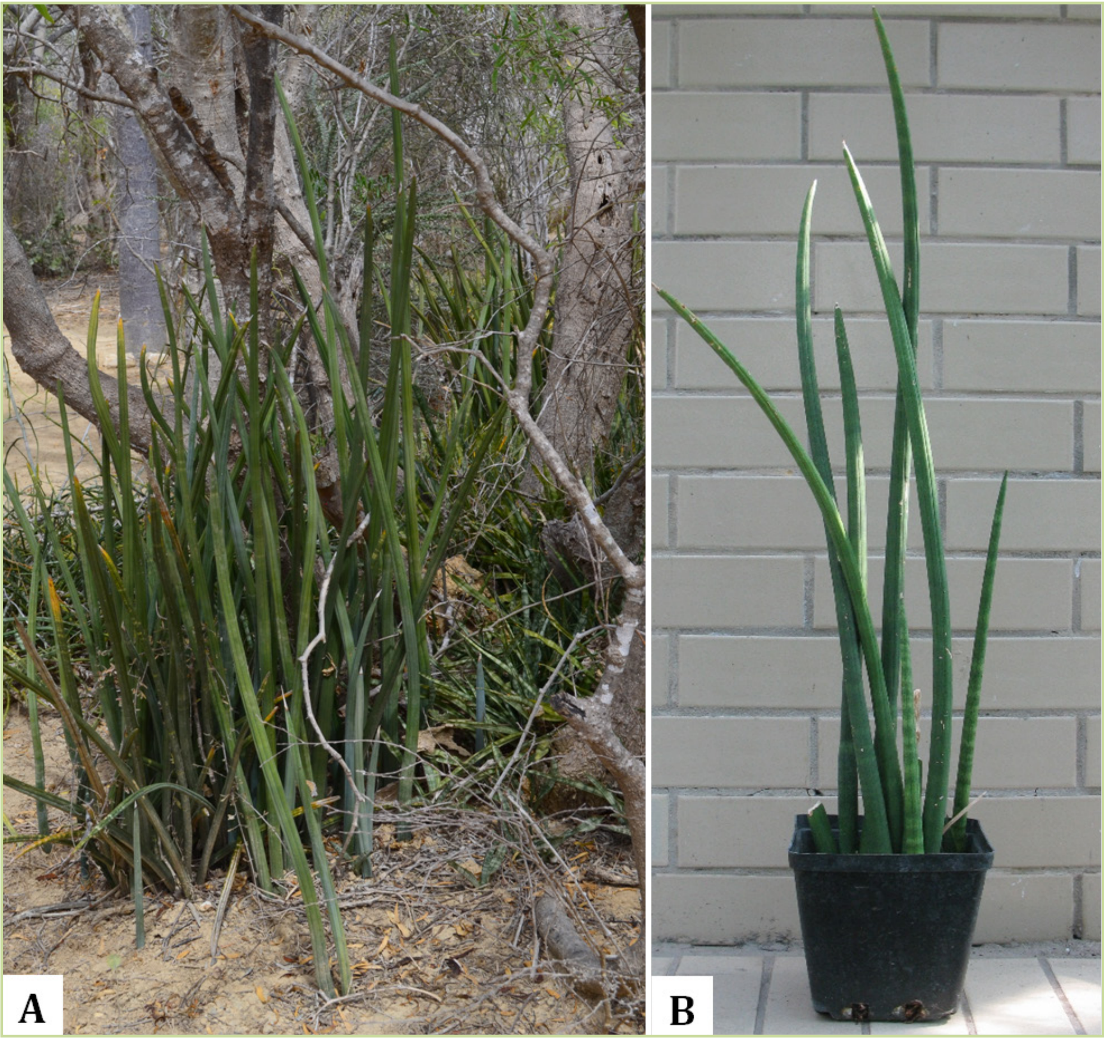

Figure 1 *Sansevieria cylindrica* Bojer ex Hook: A – the growth habit of *S. cylindrica* in semi-natural habitat, Madagascar, Toliara, d'Antsokay Arboretum (photo by Lyudmyla Buyun); B – a specimen of *S. cylindrica* cultivated under glasshouse conditions at NBG (Kyiv, Ukraine) (photo by Denis Krupoderov)

Bacterial test strain and growth conditions

For this study, a panel of organisms including *Staphylococcus aureus* ATCC 25923 (mecA negative), *S. aureus* ATCC 29213 (mecA negative, Oxacillin sensitive, weak beta-lactamase producing strain), *S. aureus* NCTC 12493 (mecA positive, Methicillin-resistant, EUCAST QC strain for cefoxitin), *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27583 were used. The cultivation medium was trypticase soy agar (Oxoid™, UK), supplemented with 10% defibrinated sheep blood. Cultures were grown aerobically for 24 h at 37 °C. The cultures were later diluted with a sterile solution of 0.9% normal saline to approximate the density of 0.5 McFarland standard. The McFarland standard was prepared by inoculating colonies of the bacterial test strain in sterile saline and adjusting the cell density to the specified concentration.

Determination of antibacterial activity of plant extracts by the disk diffusion method

Antimicrobial activity was determined using the agar disk diffusion assay (Bauer et al., 1966). Strains were inoculated onto Mueller-Hinton (MH) agar plates. Sterile filter paper discs impregnated with extract were applied over each of the culture plates. Isolates of bacteria were then incubated at 37 °C for 24 h. The plates were then observed for the zone of inhibition produced by the antibacterial activity of ethanolic extract obtained from the leaves of *S. cylindrica*. A negative control disc impregnated with sterile ethanol was used in each experiment. At the end of the period, the inhibition zones formed were measured in millimetres using the vernier. For each extract, eight replicates were assayed. The plates were observed and photographs were taken. The susceptibility of the test organisms to the plant extracts was indicated by a clear zone of inhibition around the holes containing the plant extracts and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged.

Statistical analysis

All statistical calculation was performed on separate data from each species with STATISTICA 8.0 (StatSoft, Poland) (Zar, 1999). The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (*S*) ≥15 mm, Intermediate (*I*) = 11–14 mm, and Resistant (*R*) ≤10 mm (Okoth et al., 2013).

Results and discussion

In line with the growing interest in the antibacterial potential of different plants, we examined the antibacterial properties of *Sansevieria cylindrica* leaves against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* strains. The results of antibacterial activity screening are given in the Figures 2 and 3, which clearly indicate that the extract has shown antibacterial activity against the entire tested organisms. The extract has shown better activity against *S. aureus* and *P. aeruginosa* strains compared to the *E. coli* strains. The diameters of inhibition zones were (22.5 ±1.24) mm, (20.5 ±1.3) mm, and (16.4 ±0.95) mm for *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, and *S. aureus* NCTC 12493, respectively. The extract has shown less antimicrobial activities against *P. aeruginosa*. The mean of the inhibition zone was (17.8 ±1.25) mm. Finally, the ethanolic extract exhibited mild antibacterial activity against *E. coli* [mean of inhibition zone ranged (16.8 ±0.85) mm for *E. coli* ATCC 25922 and (15.1 ±1.1) mm for *E. coli* ATCC 35218] (Figures 2 and 3).

The *Sansevieria* species are an important source of potentially useful structures for the development of new chemotherapeutic agents (Adeyemi et al., 2007; Ikewuchi et al., 2010; Andhare et al., 2012; Ikewuchi, 2012; Bhattacharjee et al., 2016). Antimicrobial screening of plant extracts with the *in vitro* antibacterial activity assay, usually, represents a starting point for antimicrobial drug discovery (Amenu, 2014). Consequently, in this study, the antibacterial activity of *S. cylindrica* leaf extract (Figures 2 and 3) was investigated against the standard Gram-positive strains: *Staphylococcus aureus* (ATCC 25923, ATCC 29213, NCTC 12493) and Gram-negative strains: *Pseudomonas aeruginosa* (ATCC 27583) and *Escherichia coli* (ATCC 25922, ATCC 35218) by the disc diffusion method. This was carried out by placing discs impregnated with test material on the surface of inoculated MH agar plates. The plates were then kept in an incubator at 37 °C for 24 hours and diameters of zones of inhibition were measured. Clear inhibition zones unravelled that the compounds showed the antibacterial activity of the antibiotic disc against bacterial strains. It was observed that controlled strain of both Gram-positive and Gram-negative strains: *E. coli*, *P. aeruginosa* and *S. aureus* were sensitive against *S. cylindrica* extract. It is concluded that plant extract possesses antibacterial activity against tested organisms. The zone of inhibition varied suggesting the varied degree of efficacy and different substances of the extract on the target strains. The antibacterial activity of the *S. cylindrica* extract may be due to the presence of various active metabolites.

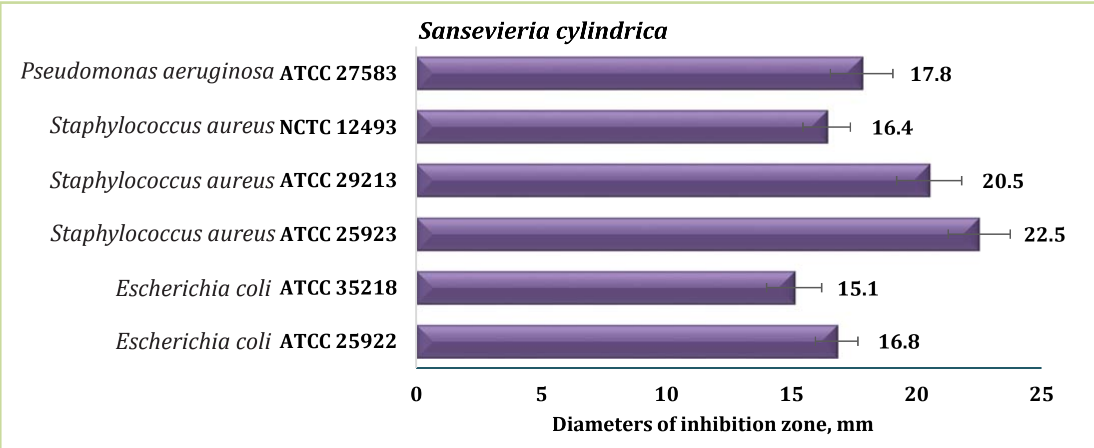

Figure 2 The mean of inhibition zone diameters of ethanolic extracts obtained from leaves of *Sansevieria cylindrica* against *S. aureus*, *E. coli*, and P. eaeruginosa (*n* = 8)

The results of the present study reinforce the importance of the analyzed plants as a source of bioactive compounds for the treatment of *S. aureus*, *P. aeruginosa*, and *E. coli* related infectious diseases. Similar results were described for other species of *Sansevieria* genus. For example, Deepa Philip et al. (2011) have carried out phytochemical analysis and antimicrobial investigation of different solvent and aqueous extracts of the leaves and rhizome of *S. roxburghiana* against a panel of clinically significant bacterial and fungal strains (*Salmonella paratyphi*, *Shigella sonnie*, *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus* spp., *Klebsiella pneumoniae*, *Proteus vulgaris* and *Cryptococcus neoformans*, *Candida albicans* and standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853). Susceptibility testing by disc diffusion assay revealed significant antimicrobial activity of methanolic and acetone extracts of leaves against Gram-positive bacteria such as *M. luteus*, *B. cereus*, *Enterococcus* spp., *S. aureus*, Gram-negative bacteria such as *P. vulgaris*, *P. aeruginosa*, *P. fluorescence*, *S. typhi*, *S. paratyphi*, *K. pneumoniae*, *S. sonnei* and *E. coli*, fungal strains *Cryptococcus* spp. and *C. albicans*. Ethyl acetate extracts of rhizomes also exhibited appreciable antimicrobial activity against most of the pathogens tested. The minimum inhibitory concentrations (MIC) of the various extracts by agar dilution method ranged from 1.0 to 8.0 $mg.ml^{-1}$. The leaf extracts exhibited better antimicrobial activity than rhizomes (Deepa Philip et al., 2011). The diethyl ether, alcohol, and acetone extracts of *S. roxburghiana* rhizome showed antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (Sheela et al., 2012). The antibacterial activity of ethanolic extract of the rhizome of *S. roxburghiana* against the four pathogenic bacteria, *S. typhi*, *P. fluorescens*, *P. aeruginosa* and *E. coli* was assessed by a zone of inhibition in the study of Poonam Sethi (2013). All the microbes were sensitive to the ethanolic extract of the plant and showed a potential activity. Maximum activity was seen in the case of *P. fluorescens* where the zone diameter was 32 mm (300 $\mu g.ml^{-1}$). The minimum inhibitory concentration study revealed that the value for the *S. typhi* and *E. coli* as 80 and 60 $\mu g.ml^{-1}$ for *P. fluorescens* and *P. aeruginosa* (Poonam Sethi, 2013).

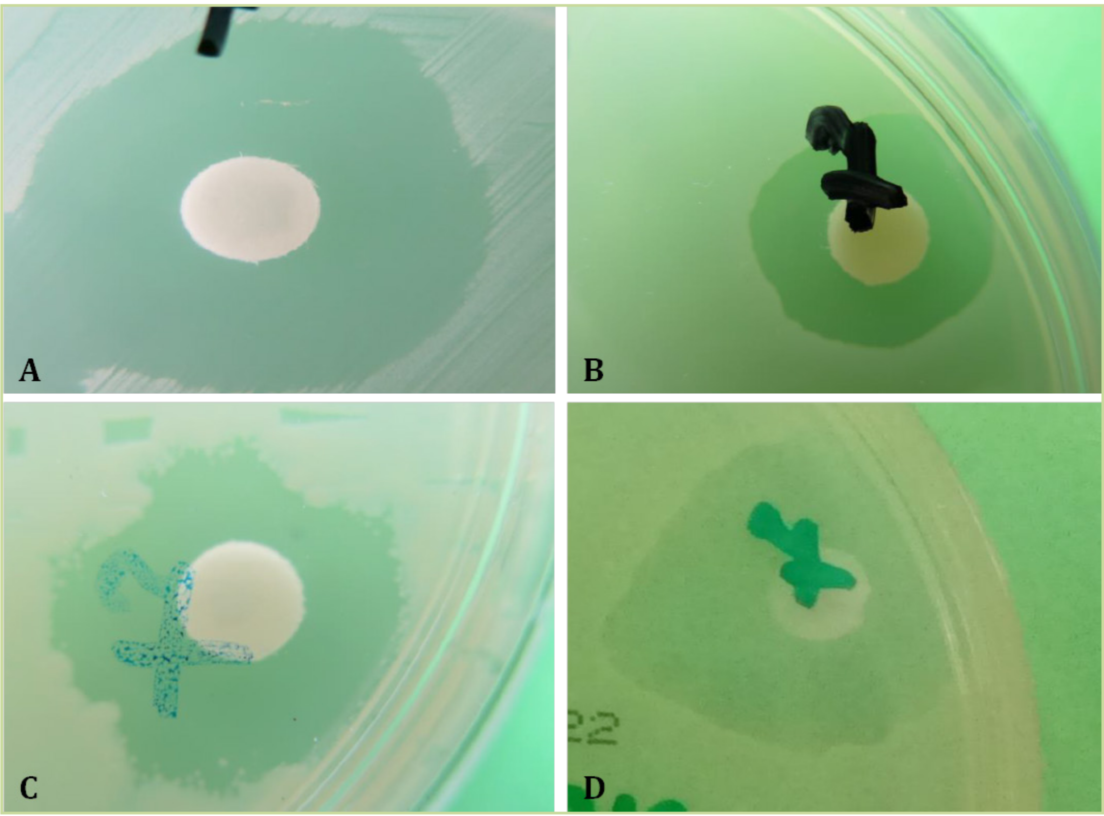

Figure 3 Antimicrobial activity of ethanolic extract obtained from leaves of *S. cylindrica* against *E. coli* ATCC 25922 (A), *E. coli* ATCC 35218 (B), *P. aeruginosa* ATCC 27583 (C), and *S. aureus* ATCC 29213 (D) measured as inhibition zone diameter

By the agar diffusion method, the ethanolic extracts from *S. fischeri*, *S. francisii*, *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, and *S. metallica* showed anti-*S. aureus* activity, evidencing that ethanol is an efficient organic solvent to be used for the extraction of bioactive plant materials. The microbial growth inhibition capacity relies on the rich variety of phytochemicals including carbohydrates, saponin, flavonoids, phenols, alkaloid, anthocyanin and cyanine, glycosides, proteins, and phytosterols (Deepa Philip et al., 2011). The phytochemical screening revealed the high presence of alkaloids in the methanolic extract of *S. roxburghiana* compared to acetone, chloroform, and ether. Flavonoids were present in ethanolic and ether extracts in moderate proportions; saponins were present in ethanolic and methanolic extracts in moderate proportions. Steroids were shown in higher proportions in methanol, chloroform and ether and moderate in acetone; terpenoids presence were was shown in chloroform and absent in all rest of the extracts. Tannins were high in acetone and methanol and moderate in ethanol and chloroform. Phenols were only in methanol fractions, while quinones were presented in methanol, chloroform, and ether in moderate levels (Kumar and Kumari, 2015).

Saponins are a diverse family of secondary metabolites (glycosylated phytoanticipins) that are found in a wide range of plant species and can be divided into three major groups, triterpenoid, steroid or steroidal glycoalkaloid, depending on the structure of their aglycones. Because they have potent antimicrobial activities it is proposed that the natural role of these molecules in plants is to confer protection against potential pathogens (Osbourn, 2003; González-Lamothe et al., 2009). Flavonoids are also well known as antibacterial agents against a wide range of pathogenic microorganism (Cushnie and Lamb, 2011; Coppo and Marchese, 2014; Xie et al., 2015). Recent advances in understanding the antibacterial properties of flavonoids were described by Cushnie and Lamb (2011). Several mechanisms of actions have been proposed for the synergistic and antibiotic resistance-modulating activity of flavonoids. For the galloyl flavan-3-ols, it has been suggested that these modulate β-lactam resistance by reducing d-alanylation of cell wall teichoic acid [resulting in inactivation of penicillin-binding protein 2a (PBP2a)], or by intercalating into the cytoplasmic membrane and inducing structural changes that result in delocalization of PBP2a. For less-studied compounds in the flavone, isoflavone, flavonol and flavolan classes, it has been suggested these increase antibiotic efficacy via multiple mechanisms of actions, i.g. through β-lactamase inhibition, efflux pump inactivation, cytoplasmic membrane destabilization, disruption of PBP2a synthesis and topoisomerase inhibition (Cushnie and Lamb, 2011).

The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases (Amenu, 2014). It was suggested, that in order to defend themselves, plants are armed with constitutive, pre-existing defence types such as cell wall barriers or pre-formed and stored antimicrobial toxins (Balmer et al., 2013).

To our mind, the scope of our current study is not limited only by searching plant-derived agents to be applied to medicine. Furthermore, we believe, that our findings are linked with the fundamental understanding of factors and mechanisms underlying both basal immunity and induced systemic resistance in angiosperms, in monocots, particularly.

Conclusions

The results proved that the extract from *S. cylindrica* exhibits a favorable antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*. Further chemical analysis of the aforementioned plant extract of *S. cylindrica* should be performed to determinate their chemical composition and identify the exact secondary metabolites responsible for the antimicrobial activity. In addition, the extract should be subjected to pharmacological evaluations with the aim of assessing its efficacy and toxicity, interactions and contraindications. Thus, the preliminary screening assay indicated that the leaves of *S. cylindrica* with antibacterial properties may offer alternative therapeutic agents against bacterial infections.

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