EVALUATION OF ANTIBACTERIAL PROPERTIES OF *XANTHIUM STRUMARIUM* L. LEAVES EXTRACTS AGAINST SOME SELECTED GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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ABSTRACT

The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight of this problem. This study is focused on exploring the antibacterial properties of *Xanthium strumarium* L plants leaves extracts in different solvents (methanol, ethanol and aqueous) was carried out against seven different bacterial strains, including four gram-positive (*Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus* sp.) and three gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) bacteria by using agar well diffusion assay (AWDA) method. Obtained results indicated that the aqueous extract gave the highest yield percentage as compared to ethanol as well as methanol extracts. The patterns of inhibition varied with the solvents extracts as well as tested bacterial strains. In the present study, samples of crude extracts displayed repressive activity against everyone treated different bacterial strains. Minimum inhibitory concentrations (MIC) a range between 12.5 to 100 mg/ml and minimum bactericidal concentration (MBC) from 12.5 to 200 mg/ml determined for the various samples of solvents extracts against the tested bacterial strains. The results showed that *M. luteus* among the gram-positive and *E. coli* among the gram-negative organisms were found highly susceptible as compared to other tested bacterial strains. An outcome of the study shows that the methanol extract had widest range of inhibitory activity as compared to the ethanol and aqueous extracts. The remarkable antibacterial activity of the leaves extracts against tested gram-positive and gram-negative bacteria suggested the possibility of employing them in drugs for the treatment of infectious diseases caused by the tested microorganisms.

***KEY WORDS*:** Antibacterial activity; *X. strumarium*; Solvents extracts; Zone of Inhibition.

INTRODUCTION

The uses of antibiotics are widespread in clinical medicine, agriculture, and veterinary promote the development of antibiotic resistances among infectious microbial strains and eventually reflects a very serious problem in the treatment of pathogenic microbes (Kapil, 2005; Bereksi, et al., 2018). Antibiotic resistance has become a serious and widespread problem in developing countries, both in hospitals and the community, causing high mortality rate in each year (Gyles, 2011). The development of antibiotic-resistant among bacterial species stems from a number of factors which include the prevalent and sometimes inappropriate uses of antibiotics, extensive uses of these agents as growth enhancers in animal feed, and increased transboundary passage of antibiotic-resistant bacteria (Lowy 2003; Djeussi et al., 2013; Elisha et al., 2017). The problem of antibiotic resistance in humans and animals will continue for a long time (Andersson and Hughes, 2011). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. Antibiotic resistance results in reduced efficacy of antibacterial drugs, making the treatment of patients difficult, costly, or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality (Wikaningtyas and Sukandar, 2016). Such a fact is cause for concern, because of number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-drug-resistant. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Consequently, new infections can occur in hospitals resulting in high mortality and the problem of microbial resistance is growing and outlook for the use of antimicrobial drugs in future is still uncertain (Nascimento et al., 2000). Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, to develop the new drugs, either synthetic or natural (Srivastava et al., 2013). The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in last decade, with more intensive studies for natural therapies. Plants have continued to be a valuable source of natural products for maintaining human health (Kavishankar et al., 2011; Kabita et al., 2015). This has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages (Lewis and Ausubel, 2006). Plants have an amazing ability to produce a wide variety of secondary metabolites, acts as antimicrobial agents (Das et al., 2010; Srivastava et al., 2013; Elisha, et al., 2017). Secondary metabolites produced by plants and microorganisms in response to external stimuli such as nutritional changes. They are widely used in the pharmaceutical industry for their remarkable structural diversity and range of pharmacological activities (Ernst, 2005).

According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. WHO estimated that 80% of the populations rely on traditional medicines, mostly plant drugs, for

their primary health care needs in developing countries (Kumar, 2014). Globally, about 85% of the traditional medicines used for primary healthcare are derived from plants. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants is found in "Rigveda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge (Gupta et al., 2010; Kumar, 2017). Plants have been used for centuries to treat infectious diseases and are considered as an important source of new antimicrobial agents (Cowan, 1999). Several works have been done to examine the antimicrobial effects of herbal plants extracts, including roots, stem, leaves or flowers (Abu-Shanab et al., 2005; Abbassi and Hani, 2012).

Xanthium strumarium L. is a cocklebur or burweed belonging to family Asteraceae and commonly found as a weed in roadsides and open dry places throughout the tropical parts of India (Oudhia, 2001; Devkota and Das, 2015; Kaur et al. 2015). It is found to be problematic in agricultural field (Figure 1). The word "Xanthium" is derived from an ancient Greek word "Xanthos" meaning yellow and "strumarium" means "cushion like swelling," with reference to the seedpods which turn from green to yellow as they ripen (later they become deep yellow to brown) (Dharmananda, 2003). It is commonly called chotagokhru due to the shape of its fruit which look likes cow's toe. This herb as such is suspected to be poisonous but the toxic substances are removed by washing and cooking (Kaur et al. 2015).

The whole plant is used as medicine. According to Ayurveda, the plant has cooling, laxative, fattening, antihelmintic, tonic, digestive, antipyretic activities and improves appetite, voice, complexion and memory. It cures leucoderma, biliousness, and poisonous bites of insects, epilepsy, salivation and fever. The plant has been reported as fatal to cattle and pigs. It is used by various Native American tribes to relieve constipation, diarrhea and vomiting. The plant is considered to be useful in treating the long-standing cases of malaria (Kaur et al. 2015).

Figure 1: Photo of *X. strumarium* L. plant

Hence, the present study was initiated to evaluate the antibacterial properties of methanol, ethanol and aqueous leaves extracts of *X. strumarium* plant against seven different bacterial strains, comprising four gram-positive and three gram-negative bacteria.

MATERIALS AND METHODS

Sources of bacterial strains

Bacterial strains, including both gram-positive and gram-negative obtained from M.D. University, Rohtak, Haryana and Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology, Chandigarh. The bacterial strains include *Bacillius subtilis*, *Micrococcus luteus* (MTCC106), *Staphylococcus aureus* (MTCC6908), *Streptococcu*s sp. (MTCC9724), *Escherichia coli* DH5α, *Pseudomonas aeruginosa* (MTCC4673) and *Salmonella typhimurium* (MTCC3224) have been selected for the present study.

Culture of bacterial strains

The bacterial strains were propagated in nutrient broth medium (5g/l peptone, 3g/l beef extract, 5g/l NaCl, and pH 7.0) incubated for 18 hr at a respective growing temperatures. Slants were prepared from the separated colonies of bacteria, stored at 4°C temperature and sub-cultured in a nutrient broth medium before testing the antibacterial activity. The chemicals were purchased from Hi-media, Mumbai, India.

Preparation of plant material

Collected fresh leaves were thoroughly washed under tap water, dried in shade for one month and then ground into coarse powdered with the help of mortar and pestle. These powders were stored in airtight brown bottles at 4°C until needed for future use.

Extraction of plant material (Maceration)

The shade dried 100 gm coarse powder of leaves of *X. strumarium* plant was immersed in 200 ml of different solvents (methanol, ethanol and aqueous) contained in 500 ml sterile conical flasks and covered with cotton wool separately. It was placed aside with intermittent shaking for one week. They were first filtered with double layered muslin cloth and then through Whatman No. 1 filter paper, and the march was discarded. The filtrate was subjected to evaporation by treating at 40°C in an oven to obtain a dried extract. Dried extract was stored at 4°C until used for further study (Atata et al., 2003; Gitika and Kumar, 2016).

Yield percentage of solvents extracts

After the drying, yield of each extraction was measured separately and the extraction efficiency was quantified by determining the weight each of the extracts and the yield percentage was calculated as dry weight/dry material weight ×100 (Parekh and Chanda, 2007).

Antibacterial activity by agar well diffusion assay (AWDA) method

The antibacterial activity of crude solvents (methanol, ethanol and aqueous) leaves extracts of *X. strumarium* against gram-positive as well as gram-negative bacterial strains were evaluated by AWDA method (Parekh and Chanda, 2007; Kumar and Gitika, 2014). Diameters of the inhibition zones were measured in millimeters (mm). For this, a well (6 mm diameter) was made with the help of a borer in cooled nutrient agar plate, overlaid with soft agar (5 ml), seeded with a target strain (~1.0 x 10^6 cfu/ml). Aliquots of the test compound (100 µl) were introduced into the well and plates were incubated for 18 hr at 37ºC. For each bacterial strain, the dissolving solvent 10% DMSO and streptomycin (50 µg/ml) were used as negative and positive controls respectively. To test the antibacterial activity of all extracts were dissolved in 10% DMSO solvent to make a final concentration 200 mg/ml.

Determination of minimum inhibitory concentration (MIC)

The MIC is the concentration giving the least inhibitory activity and below which there is no further inhibition were determined by using the Broth dilution method (Adesokan et al., 2007). Briefly, 1.0 ml of the reconstituted extract solution at a concentration of 200 mg/ml was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration of 100 mg/ml. 1.0 ml of this dilution was transferred to another test tube till the 7^{th} test tube was reached. The 8^{th} test tube did not contain any extract, but a solution of pure solvent and served as negative control. Then 1.0 ml of 18 hr grown cultures of each of bacterial strains, adjusted at ~ 1.0 x 10^6 cfu/ml was put into each tube and thoroughly mixed by vortex mixer. The tubes were incubated at 37°C for 18 hr and observed the growth in the form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection considered the MIC's value.

Determination of minimum bactericidal concentration (MBC)

MBC values were determined by removing 100 µl of bacterial suspension from the MIC positive tube as well as one above and one below the same tube, spread on nutrient agar plates and incubated at 37°C for 18 hr. After incubation, plates were examined for colony growth and MBC's value were recorded (Rahman et al., 2008; Nand et al., 2012).

Statistical analysis

The experiments were carried out in three independent sets, each consisting of three replicates. Values shown here represent mean ± standard error of the mean (SEM).

RESULTS

After complete drying, the yield percentage (gms) of *X. strumarium* leaves extracts with the various solvents (methanol, ethanol and aqueous) were measured separately and quantified the efficiency of extraction.

Outcomes of the present study, aqueous extraction gave the highest yield percentage (9.45%) followed by ethanol (8.53%) and methanol (7.55%) are illustrated in Table 1.

Table 1: Yield percentage of *X. strumarium* leaves extracts in different solvents

Solvent	**Yield percentage of extracts (gms)**		
	Weight of dry powder	Weight of dry extracts	Yield percentage
Methanol	100	07.55	07.55
Ethanol	100	08.53	08.53
Aqueous	100	09.45	09.45

X. strumarium plant leaves extracts with different solvents displayed different ranges of antibacterial potential against all the tested seven bacterial strains, comprising both the gram-positive as well as gram-negative bacteria as shown in Figure 2. In present study, methanol extracts shows inhibition against *B. subtilis* (19), *M. luteus* (22), *S. aureus* (21), *Streptococcus* sp. (18), *E. coli* (20), *P. aeroginosa* (13), and *S. typhimurium* (17). Similarly, ethanol extract produced repressive zones against *B. subtilis* (16), *M. luteus* (20), *S. aureus* (17), *Streptococcus* sp. (16), *E. coli* (18), *P. aeroginosa* (11) and *S. typhimurium* (15). Aqueous extract exhibited zone sizes towards *B. subtilis* (15), *M. luteus* (17), *S. aureus* (14), *Streptococcus* sp. (13), *E. coli* (15), *P. aeroginosa* (08) and *S. typhimurium* (13). Commercial antibiotic streptomycin used as a positive control produced higher inhibitory activity as compared to different solvents extracts used in this study with repressive zones against *B. subtilis* (23), *M. luteus* (25), *S. aureus* (22), *Streptococcus* sp. (21), and *E. coli* (25), *P.aeroginosa* (16), and *S. typhimurium* (18), whereas DMSO doesn't shows any inhibition.

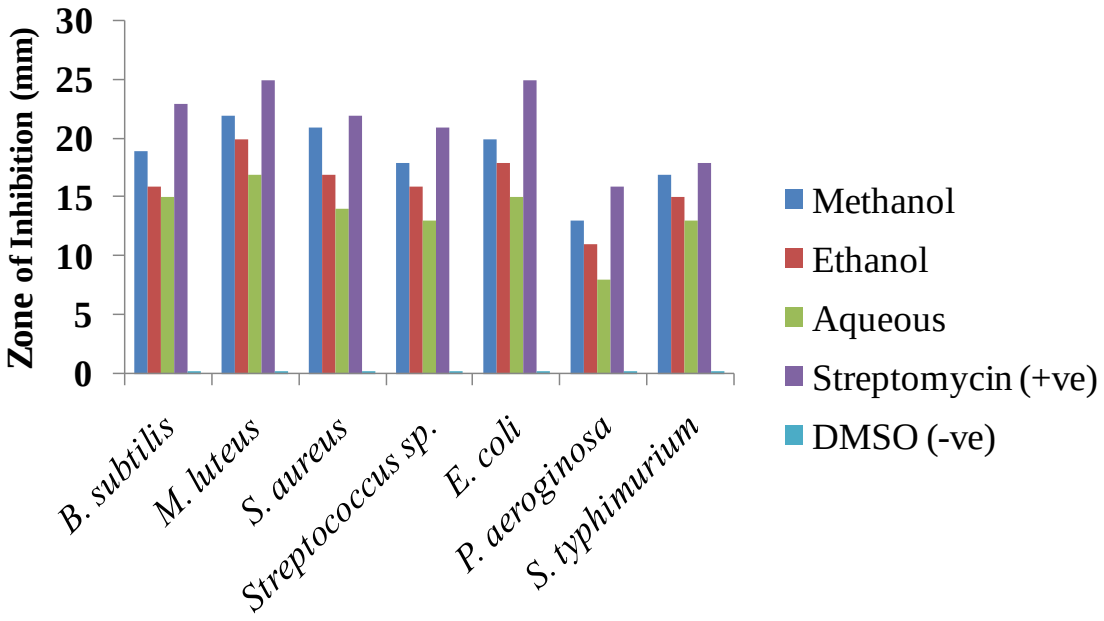

Figure 2: Antibacterial potential of *X. strumarium* leaves extracts

MIC's values for the methanol, ethanol and aqueous leaves extracts are shown in Figure 3. In this study, methanol extract exhibited 12.5 mg/ml against *M. luteus* and *S. aureus and E. coli*; 25 mg/ml against *B. subtilis, Streptococcus* sp., and *S. typhimurium*; 50 mg/ml against *P. aeruginosa*. Samples of ethanol extract possessed 12.5 mg/ml against only *M. luteus*; 25 mg/ml against *B. subtilis, S. aureus, Streptococcus* sp. and *E. coli;* 50 mg/ml against *S. typhimurium* and; 100 mg/ml against *P. aeruginosa*. Similarly, aqueous extract showed 25 mg/ml against only *M. luteus*; 50 mg/ml against *B. subtilis, S. aureus, Streptococcus* sp, *E. coli*, and *S. typhimurium*; 100 mg/ml against *P. aeruginosa*.

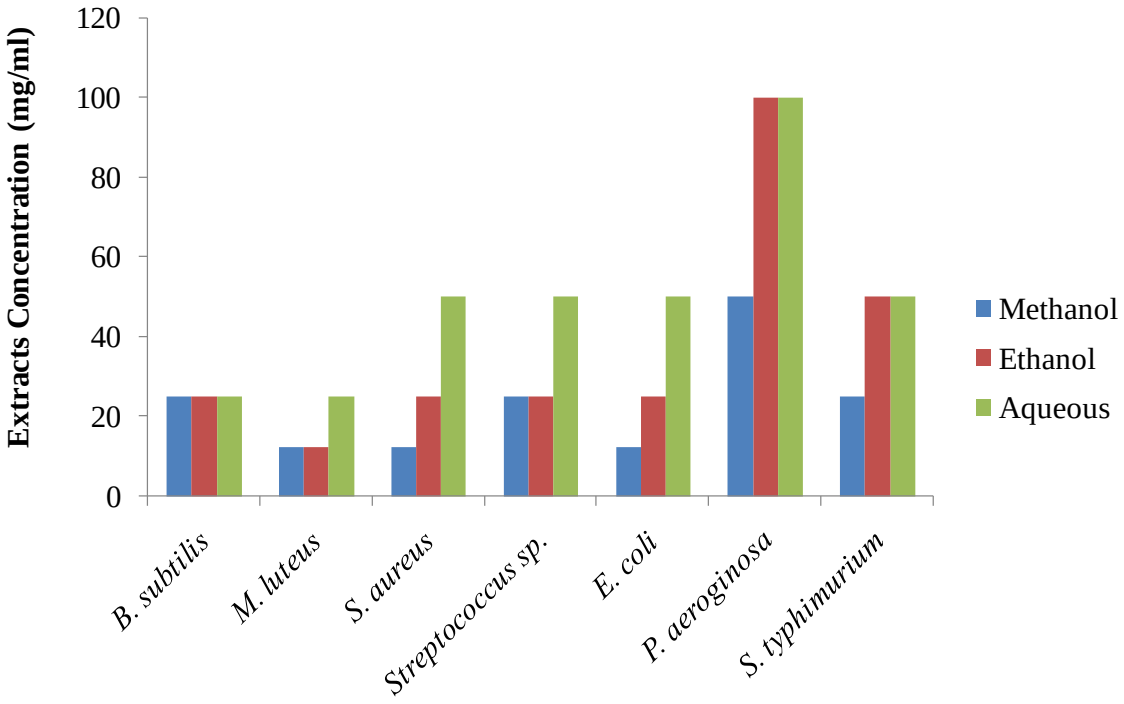

Figure 3: MIC (mg/ml) values of *X. strumarium* leaves extracts

The results of MBC's values of methanol, ethanol and aqueous leaves extracts are indicated in Figure 4. Methanol extract exhibited 12.5 mg/ml against *M. luteus;* 25 mg/ml against *B. subtilis, S. aureus*; *Streptococcus* sp., *E. coli* and *S. typhimurium*; 50 mg/ml against only *P. aeruginosa.* Ethanol samples possessed 25 mg/ml against *M. luteus, S. aureus and E. coli;* 50 mg/ml against *B. subtilis, Streptococcus* sp., and *S. typhimurium*; 100 mg/ml against *only P. aeruginosa*. Similarly, aqueous extract exhibited 25 mg/ml against *only M. luteus*; 50 mg/ml against *B. subtilis, S. aureus, Streptococcus* sp., *E. coli* and *S. typhimurium*; 200 mg/ml against only *P. aeruginosa*.

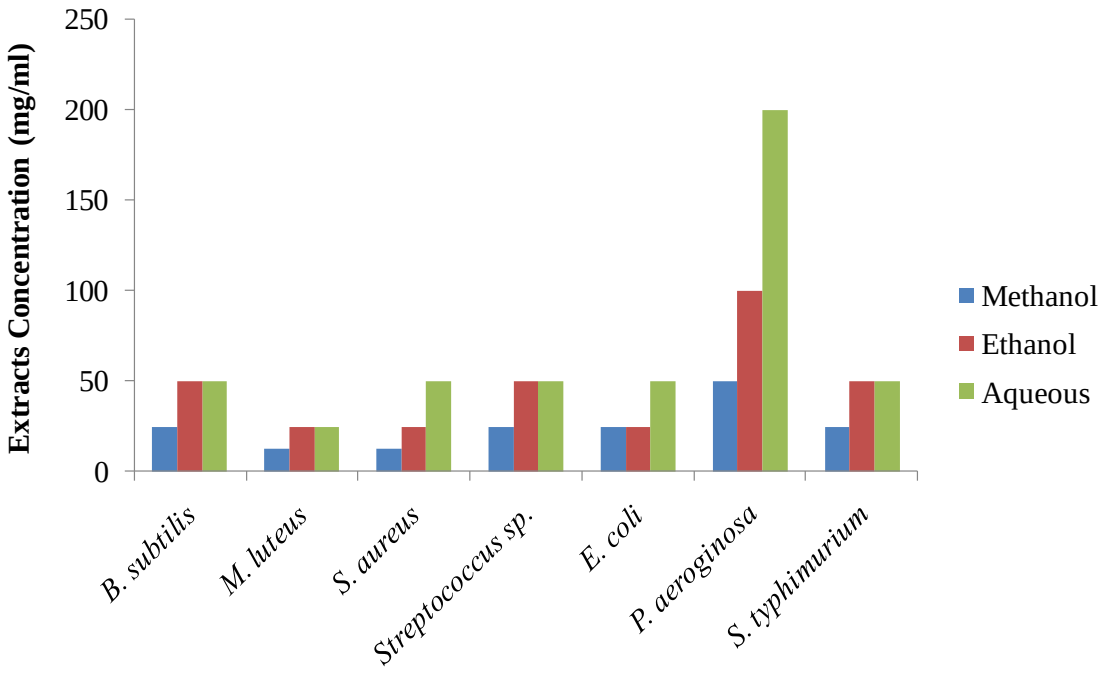

Figure 4: MBC (mg/ml) values of *X. strumarium* leaves extracts

DISCUSSION

Medicinal plants are valuable natural sources efficacious against numerous pathogenic representatives and are rich in bioactive compounds. Screening of medicinal plants used in different health care systems throughout the world remains an important resource for the discovery of novel antimicrobial agents (Weckesser et al., 2007; Palombo, 2009; Sharma et al., 2009; Pandey and Mishra, 2010; Kaur et al., 2015). Studies by different researchers have proved that plants are one of the major sources for drug discovery and development. Plants generally produce many secondary metabolites which possess chemotherapeutic, bacteriostatic, antimicrobial, antiinflammatory, anticancer, antidiabetic, antioxidant, hemolytic, larvicidal, properties and constitute a principle source of various pharmaceutical medicines (Ibrahim, 1997; Ogundipe et al., 1998; Rates, 2001; Gordon and David, 2005; Kumar et al., 2010; Shahid et al., 2013).

Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to the synthetic chemical drugs to which many infectious microorganisms have become resistant (Akinpelu and Onakoya, 2006; Chopra, 2007; Ogu et al., 2010). An extensive survey and interaction with local ethanopharmacologists, herbal drug sellers and rural negative healers revealed that native plant *X. strumarium* are widely used for treatment of various ailments of human beings as well as livestocks.

X. strumarium is a rich source of sesquiterpenoid phytochemicals with documented antibacterial activity. The *Xanthium* genus in particular is renowned for its use in alternative medicine for the treatment of infectious diseases (Wu et al. 2006; Anjoo and Ajay 2010). Its antibacterial activity has been attributed to the presence of xanthanolide sesquiterpenoids, xanthol and xanthanin. The ethnomedicinal value of *X. strumarium* has been

reported by many literatures, but there is little scientific proof for further using this plant commercially or in a more effective form. Also, it insisted to verify the traditional wisdom of local community in the use of this plant leaves as herbal drug. Though, wide range of applications focused on the antibacterial activity of leaves. For this, the yield of extraction was calculated because the crude plant extracts are generally a mixture of active and non-active compounds. A number of medicinal plants described in Ayurveda still need to be testified, according to the modern parameters to ensure their activity and efficacy. Drugs used in Ayurveda are mostly prepared by extraction with water. Therefore healers may not be able to extract all the active compound(s) (Sato et al. 1997; Khond et al., 2009; Hassan et al., 2014).

The yield percentage of medicinal plant extracts which contain bioactive metabolites vary considerably with plant species and the method or solvent used for extraction. Also, factors like age of the plant and polarity of the solvent used may have affected the yield percentage (Yahaya et al. 2012; Gitika and Kumar, 2016). In the present study, aqueous solvent extract gave the highest yield of extraction followed by ethanol and methanol, and the used various solvent extracts exhibited inhibitory activity against all the tested seven different bacterial strains comprising both gram-positive as well as gram-negative bacteria with varying degrees. In general antibacterial activity depends on the capacity of extraction of active component with varying solvents. This is in agreement with many other literatures, reported the existence of differences in percentage yield and the action of the crude extracts obtained from the same morphological part of a plant utilizing different solvents (Clark et al., 1997; Ellof, 1998; Aliero and Afolayan, 2006.; Parekh and Chanda, 2007; Khond et al., 2009; Bhandary et. al., 2012; Yahaya et al., 2012; Sahraoui et al., 2013).

After quantification of yield percentage, all the extracts have been assessed for their inhibitory activity against seven different bacterial organisms. In none of the above assay inhibition zone was not higher than the standard antibiotic streptomycin, while DMSO doesn't show any zone of inhibition. In the present investigation, samples of methanol, ethanol and aqueous extracts of *X. strumarium* displayed repressive activity against everyone treated different bacterial strains with the MIC range between 12.5 to 100 mg/ml as well as MBC values from 12.5 to 200 mg/ml against the tested microorganisms.

In one of the study, *X. strumarium* extract affected both methicillin-sensitive *Staphylococcus aureus* and MRSA, though antibacterial activity was more effective on methicillin-susceptible *S. aureus* spp. (Rad et al., 2013). However, among both types of microorganisms, gram-positive found higher susceptible for extracts as compared the gram-negative bacterial organisms, (Vlietinck et al., 1995; Rabe and van Staden, 1997; Devkota and Das, 2015). In the present study, methanol extract had widest zone of inhibition as compared to ethanol and aqueous extracts. Similar types of results have also been reported with methanol leaves extract of X. *strumarium* (Srinivas et al, 2011).

CONCLUSION

The results of present study indicated *X. strumarium* plant leaves extracts with various solvents possesses significant inhibitory activity against tested gram-positive (*M. luteus, B. subtilis, S. aureus* and *Streptococcus* sp.) as well as gram-negative bacteria (*E. coli, P. aeruginosa* and *S. typhimurium*) as a promising source of antibacterial agents. Outcomes of the present investigation are in general agreement to a certain degree with the traditional uses of this plant. The present study indicated that *X. strumarium* plant leaves extracts can be used in the medicine and will be a good source to treat and control various diseases caused by the tested bacterial organisms. Newer antimicrobials from the plant leaves extracts could also be of commercial interest to pharmaceutical companies and research institutes. Other investigations are necessary to be done on a wide range of bacteria and fungi to assess the spectrum of such plants parts extracts. Moreover, other parts of the examined plants are also needed to be assessed for their antibacterial activity. Further studies on isolation and chemical structure determination of active compounds from these extracts are necessary for their utilization in drug designing in designing and developing new drugs.

COMPETING INTERESTS

The author declared that he has no competing interests.

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