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Validation of medicinal values of traditionally used *Sonchus asper* (prickly sow thistle) leaves for the treatment of skin ailments

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ABSTRACT

The plant *Sonchus asper* has been used for the cure of skin ailments and many other disease conditions. Thus, the study was planned to compare the anti-bacterial and anti-fungal activities of aqueous and methanolic extract of *S. asper* against bacterial pathogens viz. *Staphylococcus aureus, Escherichia coli, Bacillus cerus* and *Klebsiella pneumoniae* and fungal pathogens *Candida albicans* and *Aspergillus flavus*. The findings of study indicated that the plant possesses antibacterial activity; however, it showed more inhibitory results against the Gram positive bacteria in comparison to Gram negative bacteria. Methanolic extract of *S. asper* showed activity against *E. coli* whereas *S. aureus* and *B. cerus* were inhibited by both methanolic and aqueous extracts. Bacterial isolate of *K. pneumoniae* and both the fungal species were resistant to both methanolic and aqueous extracts of *S. asper* as no zone of inhibition was observed during the experiments. The extracts of *S. asper* showed best activity against *S. aureus*, the most common pathogen of skin ailments particularly boil and wounds, thus findings of the study justified the traditional use of this plant; however, clinical validation of efficacy is and animal toxicity studies are also required to ascertain the adverse effects, if any.

Keywords: Validation, *Sonchus asper*, medicinal values, skin ailments.

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INTRODUCTION

Sonchus asper, prickly sow thistle, plants are used in traditional system for its medicinal values but are of low profile. Prickly sow thistle has been used as a pot herb since ancient period (Khan et al., 2010). As *S. asper* is used in various human disorder including wounds and burns (Hussain et al., 2008; Qureshi et al., 2009; Hussain et al., 2010) cough, bronchitis and asthma (Ahmad et al., 2006, Koche et al., 2008), gastrointestinal infection, inflammation, diabetis and cardiac dysfunction (Sabeen and Ahmad, 2009), kidney and liver disorders (Rivera and Oben,1993; Zabihullah et al*.,* 2006), reproductive disorder like impotence (erectile dysfunction) in humans (Kareru et al., 2007), jaundice (Jan et al*.,* 2009) and cancer (Sammon, 1998; Thomson and Shaw, 2002).

These conditions are produced by common bacterial pathogens as *Staphylococcus aureus, Bacillus cerus, Klebsiella pneumoniae* and *Escherichia coli.* Among them *S. aureus* is frequently part of the skin flora found in the nose and on the skin. About 20% of the human population is long-term carrier of *S. aureus,* a common skin commensal (Kluytmans et al., 1997). *S. aureus* can also cause a range of illnesses from minor skin and soft tissue infections, such as pimples, carbuncles, scalded skin syndrome, impetigo, boils (furuncles), cellulitis, folliculitis, and abscesses, to life-threatening diseases of respiratory tracts, bone, joint, endovascular to wound infections such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain,

bacteremia and sepsis. These conditions due to *S. aureus* infections are still one of the five most common causes of nosocomial infections and are observed often causing infections in postsurgical wound (Ogston, 1984). In contrast to *S. aureus, B. cerus* is responsible for a food borne illness (2 to 5%), causing severe nausea, vomiting and diarrhea (Kotiranta et al., 2000). Bacillus food borne illnesses occur due to survival of the bacterial endospores when food is improperly cooked (Turnbull, 1996). This problem is compounded when food is then improperly refrigerated, allowing the endospores to germinate (McKillip, 2000). Enterotoxins are also produced by bacterial growth, one of which is highly resistant to heat and to pH between 2 and 11; ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome (Ehling-Schulz et al., 2004).

As far as Gram negative bacteria are concerned, it is well established that the virulent strains of *E. coli* are common cause of gastroenteritis, urinary tract infections, and neonatal meningitis. Moreover, the infection due to these virulent strains mostly remains unseen or ignored being the natural habitat of gastrointestinal tracts. Sometime, virulent *E. coli* strains are also responsible for haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and Gram-negative pneumonia (Todar, 2007), whereas Klebsiella ranks second to *E. coli* for urinary tract infections in older persons (Podschun and Ullman, 1998).

Other than these bacterial pathogens, two fungal etiological agents *C. albicans* and *Aspergillus* spp are very common pathogens for animal and human infections.

As the extracts of *S. asper* are applied mostly to fresh injuries particularly on wounds and boils (Hussain et al., 2010) and its use as emollient has also been reported (Giner et al., 1993). Despite of all these potential, it is almost ignored plant with important medicinal values like wound healing and cure for boils on skin, even then in many areas prickly sow thistle are simply considered as noxious weeds (Hussain et al., 2010). Keeping all these in mind, the present study was designed to evaluate antibacterial and antifungal activities of hot aqueous and hot methanolic extracts produced from the leaves of plant *S. asper* against a group of common human pathogens of bacterial origin viz. Gram positive *S. aureus* and *Bacillus cerus,* Gram negative *E. coli and Klebsiella pneumoniae* and two fungal pathogens as *C. albicans* and *A. flavus.*

METHODOLOGY

Collection of leaves of *Sonchus asper*

The plants were collected from the premises of UP Pt. Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Gau Anusandhan Sansthan, (DUVASU) Mathura and from the village; Mangali Ghari, Post; Barauli, Baldeo, Mathura during the months of February and March of 2010 and verified prior to use by Dr. A. K. Agarwal, Professor, Department of Botany, B.S. A. College, Mathura (U.P.) for its identification and the specimen was deposited in department of Veterinary Microbiology, DUVASU, Mathura, India. Leaves of plants were collected washed with distilled water and then dried in incubator at 37°C. Dried leaves were meshed into small pieces and ground into fine powder. Dried and grinded fine powder was applied to prepare hot aqueous extract (HAE) and hot methanolic extract (HME).

Bacterial isolates

Bacterial pathogens isolated from the cases of skin ailments from the samples of human and animal wounds and boil belonging to two different groups as Gram positive *S. aureus, B. cerus and* Gram negative *K. pneumoniae* and *E. coli* were obtained from the Department of Microbiology and Immunology, UP Pt. Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Gau Anusandhan Sansthan, (DUVASU) Mathura, and used to determine *in vitro* antibacterial activity of HAE and HME of *S. asper*. All the bacterial isolates used in the study were subjected for reconfirmation by routine laboratory examinations (Kreig and Holt, 1984).

Fungal isolates

Two fungal isolate, *C. albicans as* yeast and *A. flavus* as multicellular type, isolated from the skin samples of animals were also obtained from the Department of Microbiology and Immunology, U.P. Pt. Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Gau Anusandhan Sansthan (DUVASU) Mathura, and used to determine *in vitro* antifungal activity of HAE and HME of *S. asper*. Fungal isolates used in the present study, *C. albicans and A. flavus,* were also characterized on the basis of routine laboratory methods (Kreig and Holt, 1884).

Preparation of extracts

The present study included two types of extracts as:

(a) Hot aqueous extract (HAE) of *S. asper* leaves.
(b) Hot methanolic extract (HME) of *S. asper* leaves.

Preparation of HAE of Sonchus asper leaves using Soxhlet's apparatus

50 g of dried powder of *S. asper* leaves was placed in a porous cellulose thimble. The thimble was then placed in an extraction chamber above a collection flask and the flask was filled with the 750 ml solvent (triple distilled water). The solvent was allowed to evaporate with the adjustment of boiling temperature of the solvent (100°C). The extraction process lasted for 6 to 8 h and the flask containing the solvent and extract was removed. The solvent in the flask was then evaporated and remaining material was collected and weighed and stored at 4°C.

Preparation of HME of Sonchus asper leaves using Soxhlet's apparatus

In this method, 50 g of dried powder of *S. asper* leaves was placed in a porous cellulose thimble to place in an extraction chamber above a collection flask containing Hydro-methanol solvent and rest of the method was like HAE preparation.

Hydro-methanol solvent: 30% water + 70% pure methanol

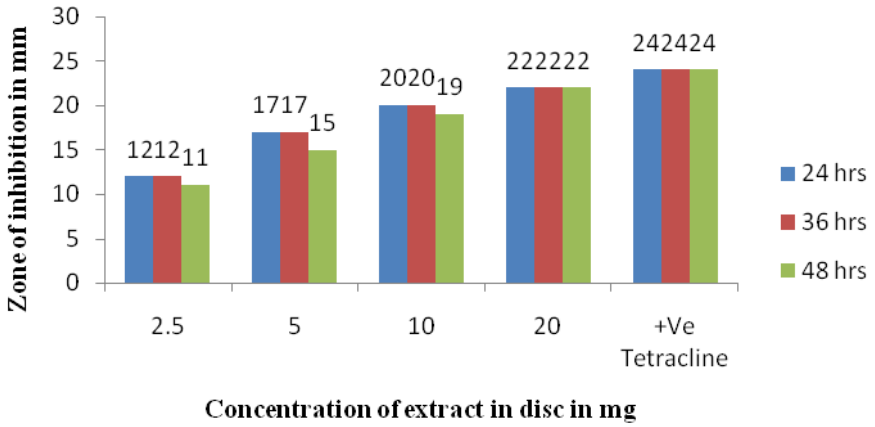

Figure 1. Effect of HAE of *Sonchus asper* leaves on *S. aureus.*

(Addition of 180 ml water in 420 ml methanol to make final volume 600 ml).

Antibacterial and antimycotic activity of *Sonchus asper* leaves

Preparation of McFarland's nephelometer for determination of bacterial concentration

McFarland's nephelometer for determination of bacterial concentration was used. For it, 1% solution of H_2SO_4 and barium chloride were used in 10 chemically cleaned standard test tubes in which first of all 0.1 ml of 1% $BaCl_2$ solution was added to the first tube and 1% $BaCl_2$ solution to remaining 9 tubes were added increasing the amount by 0.1 ml for each succeeding tube and thus the 10^{th} tube contained 1 ml of 1% $BaCl_2$ solution. Enough amount of 1% solution of H_2SO_4 was added to each tube to bring the total volume of all tubes to 10 ml.

Preparation of herbal discs

Whatman's filter paper no 1 discs of 6 mm diameter were prepared (Cruickshank, 1975) and sterilized by dry heat at 160°C for 60 min and then dipped in solution of different concentrations of HAE and HME of *S. asper* leaves. Discs were allowed to dry and stored in refrigerator for further use.

***Invitro* antimicrobial activity:** All the bacterial cultures grown freshly in broth cultures containing approximately 6.0×10^8 CFU/ml and mycotic cultures containing approximately 9.0×10^8 CFU/ml were used as an inoculum for study of antibacterial and antifungal efficacy of plant extracts. Discs containing the different concentrations of extract viz. 2, 5, 10 and 20 mg of HAE and HME of *S. asper* leaves were used to study the antimicrobial activity against bacterial and fungal cultures. Freshly grown Broth of bacterial and fungal cultures in the volume of 0.5 ml containing approximately 3×10^4 bacteria or fungi/ml was used. To assess the effect of concentration of the extract on the growth of bacteria, fungi discs containing four different concentrations of *S. asper* leaves were planted at even distance (8 to 10 mm) on nutrient agar and on SDA plates for bacterial and fungal cultures, respectively. Whereas, plain disc loaded with sterile distilled water was taken as negative control. The positive control included the discs of Tetracycline, Penicillin, Amikacin and Fluconazole (Himedia, Mumbai). The inoculated culture plates after placing discs over them were incubated at 37°C up to 72 h. The antimicrobial activity of extract was measured in mm by scale considering the area marked by the zone of inhibition of bacterial/ fungal growth around the disc at 24, 48 and 72 h intervals. As a thumb rule, all the discs were tested for sterility prior to use.

RESULTS

Effects of aqueous extract: Hot aqueous extract of *S. asper* revealed a dose dependent effect against all the bacteria except *K. pneumoniae.* The measurement of zone size ranged from 12 to 22 mm against *S. aureus.* The findings of the study were comparable to the antibiotic disc of Tetracycline which showed zone size of 24 mm and this effect of zone was maintained even after 48 h. The effect was dose dependent as it increased with the increase of concentration of extract on disc and the disc with the conc. of 20 mg/disc revealed maximum zone of inhibition (Figure 1). During the study, it was observed that HAE of *S. asper* was less effective against *B. cerus* as the discs with the concentration of 2.5 and 5 mg revealed no zone of inhibition even after the incubation of 48 h. Discs of 10 and 20 mg concentration revealed zone of inhibitions in the range of 8 to 18 mm. It is less than the antibiotic disc of Penicillin which showed zone size of 25 mm. These effects of inhibiting the bacterial growth started reducing after 48 h of incubation (Figure 2). There was no effect of HAE of *S. asper* on *K. pneumoniae.* It revealed no zone of inhibition even after 48 h of incubation. Effect of HAE of *S. asper* was not significant against *E. coli.* Discs with the concentration of 2.5 and 5 mg showed no zone of inhibition even after the incubation of 48 h. Discs of 10 and 20 mg concentration revealed zone of inhibitions with the size in the range of 11 to 19 mm. It is less than the antibiotic disc of Amikacin which showed zone size of 40 mm. Moreover, the zone size increased up to 36 h but then started reducing after 48 h of incubation (Figure 3). HAE of *S. asper* showed no antimycotic activity against both unicellular *C. albicans* and multicellular *A. flavus.*

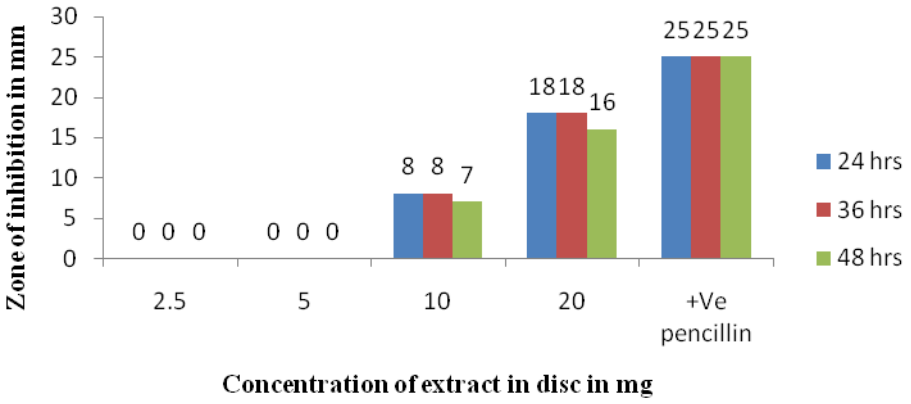

Figure 2. Effect of HAE of *Sonchus asper* leaves on *Bacillus cerus*.

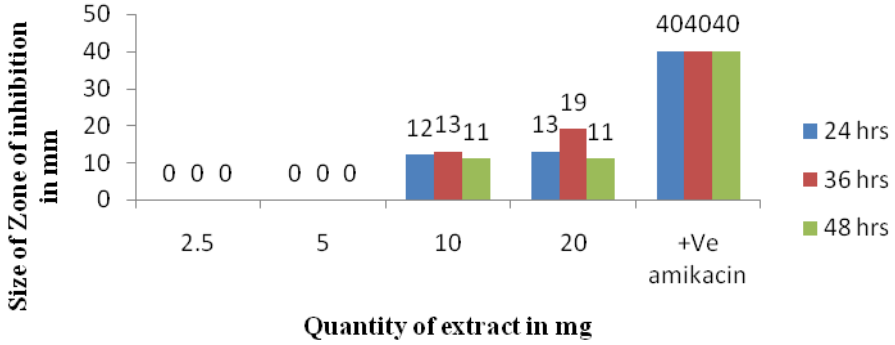

Figure 3. Effect of HAE of *Sonchus asper* leaves on *E. coli.*

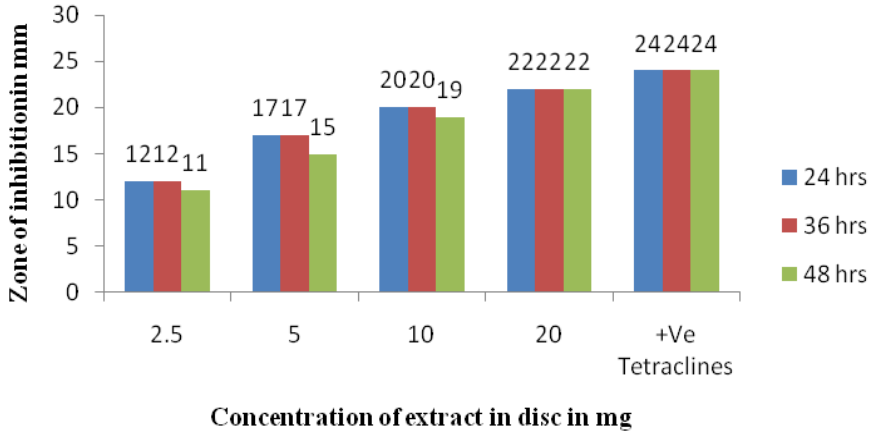

Figure 4. Effect of HME of *Sonchus asper* leaves on *S. aureus.*

Effects of hydro methanolic extract

Zone size ranged from 12 to 22 mm against *S. aureus.* These findings were comparable to the positive control antibiotic disc of Tetracycline which showed zone size of 24 mm. Moreover, the effect of zone was maintained even after 48 h. These findings suggested that the effect was dose dependent as it increased with the increase of concentration of extract on disc and was maximum with the concentration of 20 mg/disc (Figure 4). HME of *S. asper* was less effective against *B. cerus.* Discs with the concentration of 2.5 and 5 mg showed no zone of

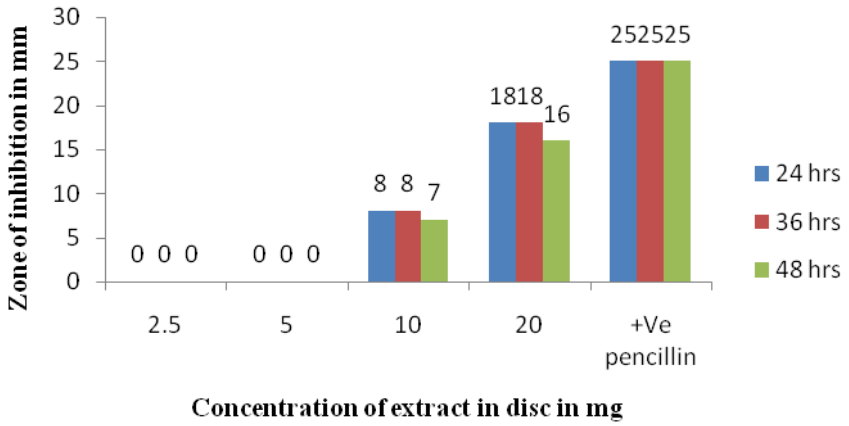

Figure 5. Effect of HME of *Sonchus asper* leaves on *Bacillus cerus*.

inhibition even after the incubation of 48 h. Discs of 10 and 20 mg concentration revealed zone of inhibitions and zone size ranged from 8 to 16 mm in comparison to the antibiotic disc of Penicillin which showed zone size of 25 mm. Similar to the findings with HAE, the effect of zone started reducing after 48 h of incubation (Figure 5). Discs of 2.5, 5, 10 mg concentration showed no zone of inhibition. Only the discs of 20 mg showed a mild zone of inhibition with the size of 11 mm up to 36 h and 10 mm after 48 h. There was no effect of HME of *S. asper* on *K. pneumoniae.* It revealed no zone of inhibition even after 48 h of incubation. HME of *S. asper* showed no antimycotic activity against *C. albicans* and *A. flavus.*

DISCUSSION

The use of *S. asper* as a pot herb by Western Indians, as salad and pot herb by Romans and soups of its tender leaves by Europeans itself indicates its application in human life (Guil-Guerrero et al., 1998). Asian counterparts use its top and leaves after steaming which make a palatable and nutritious leaf vegetable (Khan et al., 2010). Among many species of genus *Sonchus, S. asper* is very less explored; whereas species like *Sonchus oleraceae* have well established good medicinal properties due to the presence of flavonoids (Giner et al., 1993; Manez et al., 1994) glycosides (Shimizu et al., 1989), ascorbic acid and carotenoids. These carotenoids are reported to have antioxidant, anticancer, anti-inflammatory properties (Guil-Guerrero et al., 1998). Moreover, prickly sow thistle has been used as pot herb since ancient time (Hutchinson et al., 1984). The bacterial and mycotic pathogens used in the present study were selected on the basis of their involvement in human and animal skin ailments.

Both the extracts revealed similar inhibition patterns which was almost equal to antibiotic Tetracycline; thus, it justifies the use of *S. asper* in skin affections. These findings were in concurrence to the findings of others, while studying the methanolic, water, butanolic, hexane and ethanolic extracts of *S. asper* reported that *S. aureus* was inhibited (MIC) by butanolic extract (5 µg/ml), methanolic extract (2.5 µg/ml) and aqueous extract (5 µg/ml) (Sabeen and Ahmad, 2009).

Different parts of the *S. asper* are used for different function as leaves and roots of the plant are used in indigestion and as a febrifuge. The roots of the plants act as a vermifuge and the stems are recommended as a sedative and tonic (Ambasta, 1992). As most of conditions produced by *B. cerus* are related to toxin and are due to the bacterial growth in food and food products so the extracts of *S. asper* might be suggested as preservative to prevent the bacterial growth. During the present study both HAE and HME of *S. asper* showed zone of inhibition with the concentration of 10 and 20 mg. However, plant extracts in the concentration of 2.5 and 5mg inhibited no growth. The zones of inhibition by the HAE and HME were almost similar and persisted up to 36 h. Spore forming organisms Bacillus were less sensitive in comparison to *S. aureus.* These findings are in contrast to the findings of workers (Sabeen and Ahmad, 2009) who found no effect of HAE on the bacteria *B. subtilis*, however they reported that *B. subtilis* was inhibited by methanolic and ethanolic extracts (1 µg/ml) of *S. asper.*

To compare the effects of *S. asper* extracts on Gram negative bacteria in comparison to Gram positive bacteria *E. coli* and *K. pneumoniae* were considered in the present study. The extracts of both the type as HAE and HME of *S. asper* produced limited effect on two Gram negative bacterial pathogen. The effects of HAE was better than HME as in case of HAE, 10 and 20 mg concentration revealed the zone of inhibition although, the size of the zone was not large enough to be considered as sensitive and HME produced only a mild zone of inhibition even with the concentration of 20 mg. Similarly Khan and coworkers (2010) also reported *E. coli* sensitive to methanolic extract of *S. asper* with the MIC of *E. coli* 5 µg/ml. However, in contrast to the present study

it was not found sensitive to HAE of *S. asper* leaves.

K. pneumoniae growth was not inhibited by both HAE and HME of *S. asper* plant as there were no visible zones of inhibition with any of the concentration. These findings are in contrast to the observations of others (Khan et al., 2010) who reported that *K. pneumoniae* growth was inhibited (MIC) by hexane extract, methanolic extract (1 µg/ml) and ethanolic extract (5 µg/ml). However, these variations might be due to the quality and different active principle of the extract (Mahima et al., 2012). As new antibiotic resistant strains of *K. pneumoniae* are appearing (Tiwari et al., 2012) and it is increasingly found as a nosocomial infection (Tiwari et al., 2012) this difference might be due to the difference of strains.

This difference in sensitivity of Gram positive and Gram negative bacteria might be due to remarkable difference in the cell wall composition of Gram negative and Gram positive bacteria (Vashney et al., 2012). Particularly in case of Gram negative bacteria cell wall is rich in lipopolysaccharide which may be a cause of difference of sensitivity in Gram positive and Gram negative bacteria. The Klebsiella are capsulated organisms and capsule always play important role as antiphagocytic factor, it may be a cause of resistance against both types of extracts as HAE and HME of *S. asper*.

In the present study, the anti fungal activities against *C. albicans* and *A. flavus* were assessed with HAE and HME of *S. asper.* It produced no results against both of fungi. It may be because of the cellular composition of fungi which have chitin in their cell wall which is not found in case of bacteria (Vashney et al., 2012).

Thus, the results produced in the present studies are in the supports of these workers as study revealed that both HAE and HME of *S. asper* are effective against bacterial pathogens present in routine infections. However, there was no effect against mycotic pathogen. Interestingly, both the extracts were not effective against *K. pneumoniae* organisms. Similarly, HME of *S. asper* also showed very mild effect against *E. coli* organisms. This variation in sensitivity of pathogens varied pathogen to pathogen and also depends upon the type of extract and concentration of extract used. However, its role in these diseases is to be validated. On the basis of the findings of the study, it can be concluded that the topical use of HAE and HME against disease conditions produced by different pathogens as *S. aureus and B. cerus* can be recommended for the cure of these ailments. It is in concurrence to use of this herb as the plant extract is applied to fresh injuries and preparations of the plant parts are pounded and effectively applied to wounds and boils.

Conclusion

This study supports the traditional use of *S. asper* on skin wounds and boils as the extracts showed inhibitory effect against *S. aureus*, the major pathogen of skin ailments. Still, there is a need to integrate traditional knowledge of *S. asper* extracts into the modern medicine practices. In present scenario, this requires clinical validation of *S. asper* extracts by conducting controlled clinical trials. The methods used for clinical validation for modern medicines must be applied to prove the safety and efficacy of the finished herbal products of *S. asper* extracts. While initiating a trial, the design and the scope of the studies should be in accordance with traditional use and in consultation with the traditional medical practitioners as the major hinderance in the amalgamation of herbal medicines into modern medical practices is the lack of scientific and clinical data, and better understanding of efficacy and safety of the *S. asper* herbal products. The traditional knowledge of historical use provides the source to study the specific plant species with potential to be used in a particular disease although experimental and clinical validation of efficacy, though a systematic approach is required for *S. asper* as is one in modern medicine; animal toxicity studies are also required to establish the potential adverse effects.

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