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Original article

Evaluation of antimicrobial potential of root extract of *Asparagus racemosus* Willd. and bark extract of *Juglans regia* L. against pathogenic bacterial isolates

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Abstract

The aim of the present research work was to investigate the antimicrobial potential of *Asparagus racemosus* Willd. and *Juglans regia* L. against certified pathogenic microorganisms; *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pneumoniae*, using agar well diffusion method. Aqueous extract of *A. racemosus* root was more effective among others and maximum inhibition was observed against *S. typhi* and *E. coli* (18 mm at 4 mg/ml). In case of *J. regia*, bark acetone extract was found most effective with highest activity observed against *S. aureus* (24 mm at 4 mg/ml). The minimum concentration of 0.4 mg in case of (*A. racemosus*) and 0.6 mg for (*J. regia*) were required to inhibit the growth of *P. aeruginosa* and *S. aureus*, respectively. Based on the initial screening results with *A. racemosus* and *J. regia* plant extracts, it can be concluded that they have the potential to be used as alternate to synthetic medicines but only after intensive research and development and clinical trials. Moreover, if successful, this will provide a resource which is relatively cheaper, safer to use and with less or no side effects.

Key words: *Asparagus racemosus* Willd., *Juglans regia* L. MIC, antimicrobial activity, herbal medicine

1. Introduction

Medicinal plant-based drugs owing to the advantage of being simple, effective and exhibiting broad spectrum activity, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003; Dogra, 2014; Devi, 2016). Plants are the richest repository of drugs for traditional medicines, modern medicines, folk medicines, pharmaceutical intermediates and chemical entities (Hammer *et al.*, 1999; Kashayap, 2017). It is important to mention that traditional medicinal systems are at a transitional stage in the development of modern medicines in developing countries. Therefore, use of neglected and little known medicinal and aromatic plants must be promoted and encouraged at regional as well as global levels for the betterment of mankind (Rizwana *et al.*, 2002). Plants produce low-molecular-weight ingredients, *i.e.*, secondary metabolites for their protection against pests and diseases, for the regulation of their growth, or as pigments, essence, or odor (Perez-Vizcaino *et al.*, 2006).

The Himalayan region represents the richest storehouse of medicinal plants. The climatic conditions prevailing in the region maintain an ideal habitat for the natural growth of variety of medicinal plants and herbs (Kumar *et al.*, 2013). According to World Health Organization (WHO) estimates, more than 80% of the people in developing countries depend on the traditional medicine for their primary health needs (Sharma and Pandey, 2009; Subramoniam, 2014; Biradar, 2015; Rajeshwer, 2015; Ansari, 2016). The effectiveness of plant extracts against various pathogenic microbes can also be enhanced through the green synthesis of silver nanoparticels using biotechnological interventions (Thakur *et al.*, 2017). *A. racemosus* and *J. regia* are two very important plants growing in Himalayan region and having immense medicinal value.

A. racemosus (Satavar, Shatavari or Shatamull) belonging to family Asparagaceae and commonly found throughout India, grows 1-2 metres tall in gravelly, rocky soils at 1,300-1,400 meters elevation (Freeman, 2009). *A. racemosus* (Shatavari) “curer of a hundred diseases” has been recommended in Ayurveda for the prevention and treatment of gastric ulcers, dyspepsia, as a galactogogue, for nervous disorders, inflammatory diseases, bronchitis, pneumonia, syphilis and other veneral diseases (Singh *et al.*, 2003). Past efforts to explore the antibacterial potential of methanolic extract of roots and leaves of *A. racemosus* indicated their utility as broad spectrum antibacterial substances, due to their increased efficacy against various Gram-positive and Gram-negative bacteria (Ganesan *et al.*, 2015; Patel and Patel, 2013).

J. regia, a species from family Juglandaceae, has been used in traditional medicines from ancient times. This royal species mostly found in the Kashmir region of Himalayas, having vast biodiversity. These species grow up to 25-35 m in height (Muzaffer and Paul, 2018). All parts of the plant, *i.e.*, roots, stem, bark, leaves, seeds and seed oil are medicinally important, being depurative, antihelmintic, laxative, detergent, astringent, diuretic, anticancerous and antimicrobial. The high concentrations of ethanolic and aqueous bark extracts of *J. regia* have been reported earlier to be effective against *S. sanguis*, *S. mutans*, *S. salivarius*, and *S. aureus*

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(Zakavi *et al*., 2013). It has also been reported that leaves of *J. regia* contain monoterpenes and sesquiterpenes and bark contains ketones like juglone, regiolone, sterol and flavonoids which also possess antimicrobial activity (Chopra *et al*., 1986).

The evaluation of plants for their potential application based on their medicinal properties is important for modern day medicine as the widespread and long-term use of antibiotics has led to emergence of multi drug resistant strains, besides several side effects. The adverse effects of these synthetic drugs can be overcome by using traditional or herbal formulations which are safe, efficacious and multifunctional (Jinukuti and Giri, 2013; Badar *et al.,* 2012). Further, the development of herbal medicines based on ethnomedical leads is relatively easier in comparison to synthetic drugs (Jinukuti and Giri, 2015). Therefore, the present work was undertaken with major focus on the study of medicinal properties of two very important plants, used by the herbal practitioners successfully and to find whether the etiologic agent is resistant or sensitive to the natural antimicrobial agent present in these plant extracts, being tested, so that the active compounds responsible could be used as the source of antimicrobial agents for the synthesis of traditional medicines.

2. Material and Methods

2.1 Plant materials

A. racemosus roots were collected from Gaggal area of Kangra district and *J. regia* bark was collected from Chamba district of Himachal Pradesh during the month of March, 2014. The samples were washed with fresh water, dried in shade for about 3 to 4 weeks, powdered using pestle and mortar and stored in air tight container until further use.

2.2 Clinical isolates

The clinical isolates, *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Streptococcus pneumonia* and *Pseudomonas aeruginosa* were obtained from Department of Microbiology, Indira Gandhi Medical College and Hospital (IGMC), Shimla, Himachal Pradesh.

2.3 Antimicrobial agent

The antibiotics were used as positive control to study the effect of plant extracts so as to evaluate their comparative efficacy against the selected pathogenic isolates.

Table 1: Antibiotics used as positive control

Microorganism used	Antibiotics
Escherichia coli	Tetracyclin
Klebsiella pneumoniae	Ciprofloxacin
Salmonella typhi	Chloramphenicol
Staphylococcus aureus	Ciprofloxacin
Streptococcus pneumoniae	Erythromycin
Pseudomonas aeruginosa	Gentamycin

2.4 Plant extracts

The powder was stored in air tight container until further use. Cold percolation method of extraction was used for the preparation of plant extract. In this method, powdered plant material was used for extraction using different solvents, *viz*., petroleum ether, chloroform, acetone, methanol and water as per the method detailed by Rosenthaler (1930). Dried powdered samples were added into the respective solvent in the ratio of 1:10 in 250 ml flask and kept on a rotary shaker (150 rpm) for 48 h at 35°C. The resultant extract was filtered through Whatmann No. 1 filter paper and allowed to evaporate under room temperature. The residual material from the first extraction was further extracted in the next solvent. For every extraction, from every 10 gm sample used, about 600-700 mg of plant extract could be obtained which was stored at 4°C until further use.

2.5 Stock solution

The stock solutions of different plant extracts were prepared to get final concentration of 100 mg/ml, using 10% dimethyl sulfoxide (100 µl DMSO diluted to 900 µl distilled water).

2.6 Determination of antimicrobial activity

The effect of plant extracts on the bacterial strains (Clinical isolates) was measured by agar well diffusion method. Medium was prepared by dissolving 33.9 g of commercially available Muller Hinton Agar (Hi Media) in 1000 ml of distilled water. The autoclaved medium was poured into the petriplates and 20 to 30 µl of each inoculum was spread on selective plates and labelled properly. Sterile borer of 6 mm diameter was used for gently boring wells in solidified petriplates. Crude extracts and controls were added into the respective wells after that the plates were incubated at 37°C for overnight incubation. The extent of clearing zone was taken as a criterion for the determination of antimicrobial potential. The minimum concentration of plant extract required to inhibit the growth of microorganism was checked by Resazurin dye method (McNicholl *et al*., 2006). The MIC value of the most effective extract, *i.e*., methanolic extract was determined against all pathogenic microbes using decreasing concentrations of methanolic extract in the range of 0.2 mg/µl to 0.8 mg/µl. The MIC in case of each extract was determined after 24 h of incubation at 37°C.

3. Results

Effect of various extracts of *A. racemosus* on pathogenic isolates.

3.1 Petroleum ether extract

Petroleum ether extract of *A. racemosus* showed activity only against *S. aureus*. The zones of inhibition were 7 mm, 8 mm, 9 mm and 10 mm at the concentrations of 10 µl, 20 µl, 30 µl and 40 µl, respectively that were lower in comparison to positive control, *i.e*., 20 mm (Figure 1, Figure 4a). No activity was observed against other bacterial isolates.

3.2 Chloroform extract

The chloroform extract of *A. racemosus* showed maximum activity only against *S. aureus*. The zones of inhibition were 9 mm, 10 mm, 11 mm and 12 mm at the concentrations of 10 µl, 20 µl, 30 µl and 40 µl, respectively. The activity of chloroform extract was lower than the positive control, *i.e*., 21 mm. No inhibitory effect on the growth of other bacteria was observed (Figure 1, Figure 4b).

3.3 Methanolic extract

Methanolic extract of *A. racemosus* showed inhibitory effect on the growth of *P. aeruginosa*. The zones of inhibition were 10 mm, 12 mm, 16 mm and 17 mm at the concentrations of 10 µl, 20 µl, 30 µl and 40 µl, respectively (Figure 2, Figure 4c). The inhibitory effect of methanolic extract on *P. aeruginosa* was quite comparable with the positive control.

3.4 Aqueous extract

Aqueous extract of *A. racemosus* showed good inhibitory effect on the growth of *S. typhi*, *E. coli* and *S. pneumoniae*. The inhibitory effect of aqueous extract was quite comparable with the inhibitory effect of positive control in case of *E. coli* and *S. pneumonia* (Figure 3, Figures 4d, 4e).

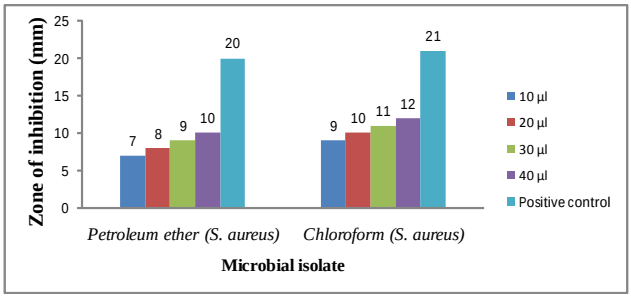


Figure 1: Antibacterial activity of root extract of *A. racemosus* against *S. aureus*.

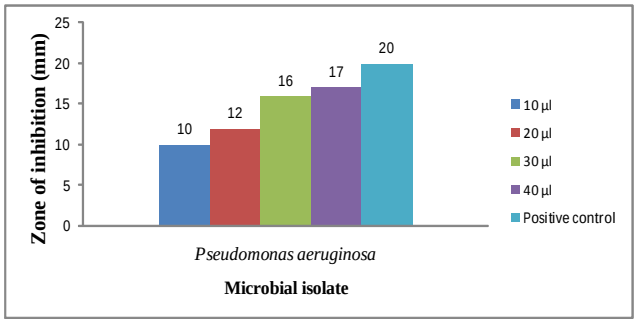


Figure 2: Antibacterial activity of methanol extract of *A. racemosus*.

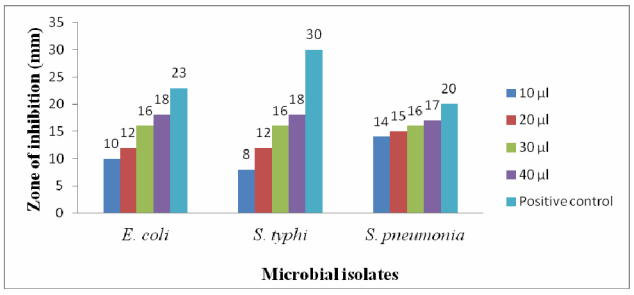
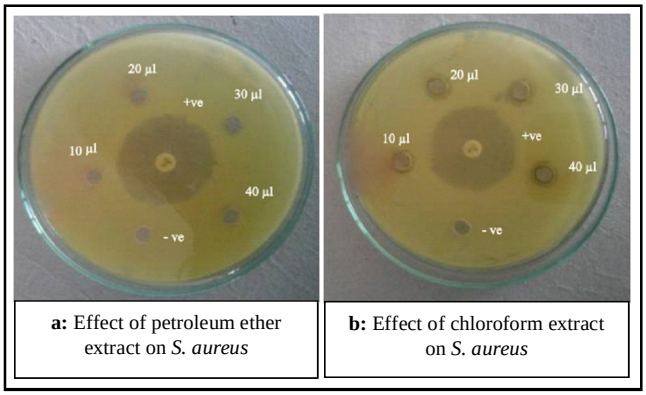
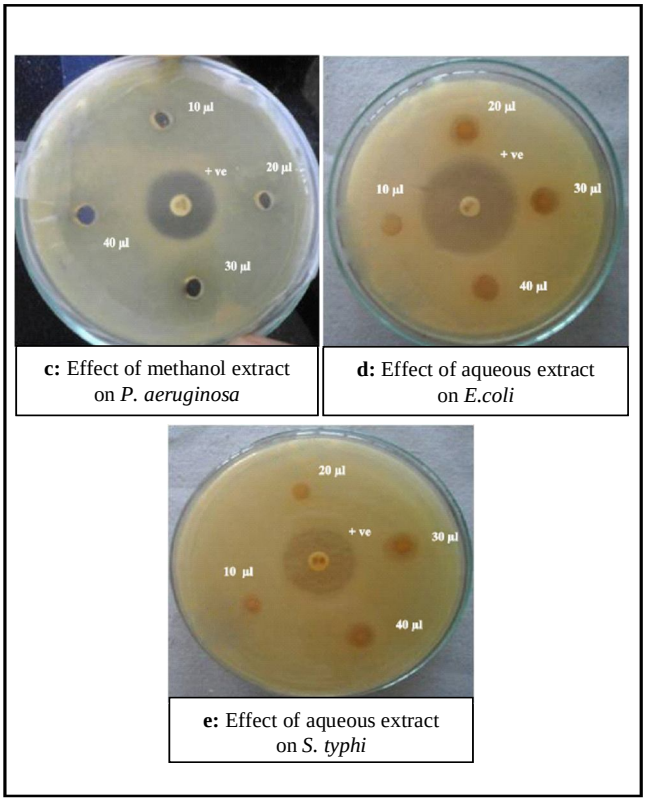


Figure 3: Antibacterial activity of aqueous extract of *A. racemosus*.



a: Effect of petroleum ether extract on *S. aureus*

b: Effect of chloroform extract on *S. aureus*



c: Effect of methanol extract on *P. aeruginosa*

d: Effect of aqueous extract on *E.coli*

e: Effect of aqueous extract on *S. typhi*

Figure 4: Effect of *A. racemosus* root extract on pathogenic bacteria.

3.5 Effect of various solvent extracts of *J. regia* on pathogenic isolates

3.5.1 Acetone extract

The effect of acetone extract of *J. regia* was observed against selected bacterial isolates. The maximum activity was observed in case of *S. aureus*, the zones of inhibition were 12 mm, 18 mm, 20 mm and 24 mm with 10 µl, 20 µl, 30 µl and 40 µl extracts, respectively (Figure 5, Figures 7a, b, c). In case of *E. coli* and *S. typhi*, inhibition was observed at higher volume of plant extract (30 µl and 40 µl), the zones of inhibition were (8 mm and 10 mm). The effect of plant extract was quite comparable with positive control in case of *S. aureus*.

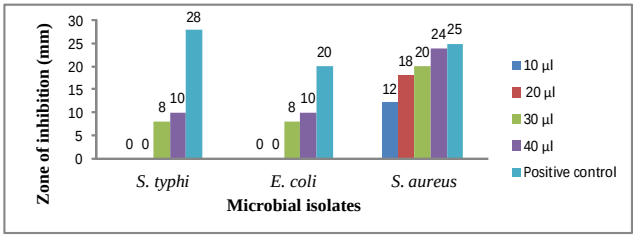


Figure 5: Antibacterial activity of acetone extract of *J. regia*.

3.5.2 Methanolic extract

Methanolic extract of *J. regia* showed inhibitory effect against both, *S. aureus* and *E. coli* but effect was much pronounced against *S. aureus* with 22 mm zone of clearance while with positive control, it was 26 mm. The zones of inhibition against *S. aureus* were 14

mm, 16 mm, 18 mm and 22 mm at the volume of 10 µl, 20 µl, 30 µl and 40 µl of extracts, respectively. In case of *E. coli*, inhibition zones were 8 mm, 9 mm, 10 mm and 13 mm at 10 µl, 20 µl, 30 µl and 40 µl volume of extracts, respectively (Figure 6, Figures 7d, e). The positive control was more effective in comparison to the crude extract especially in case of *E. coli* whereas in case of *S. aureus*, the relative efficacy was quite comparable.

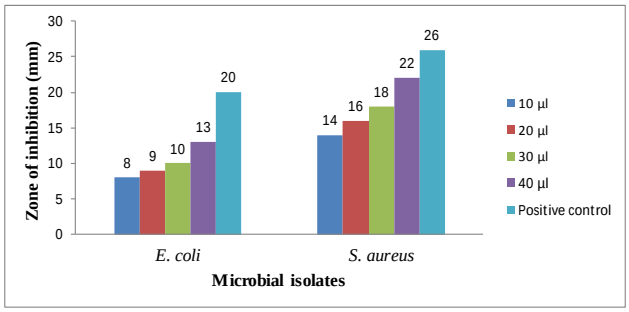


Figure 6: Antibacterial activity of methanol extract of *J. regia*.

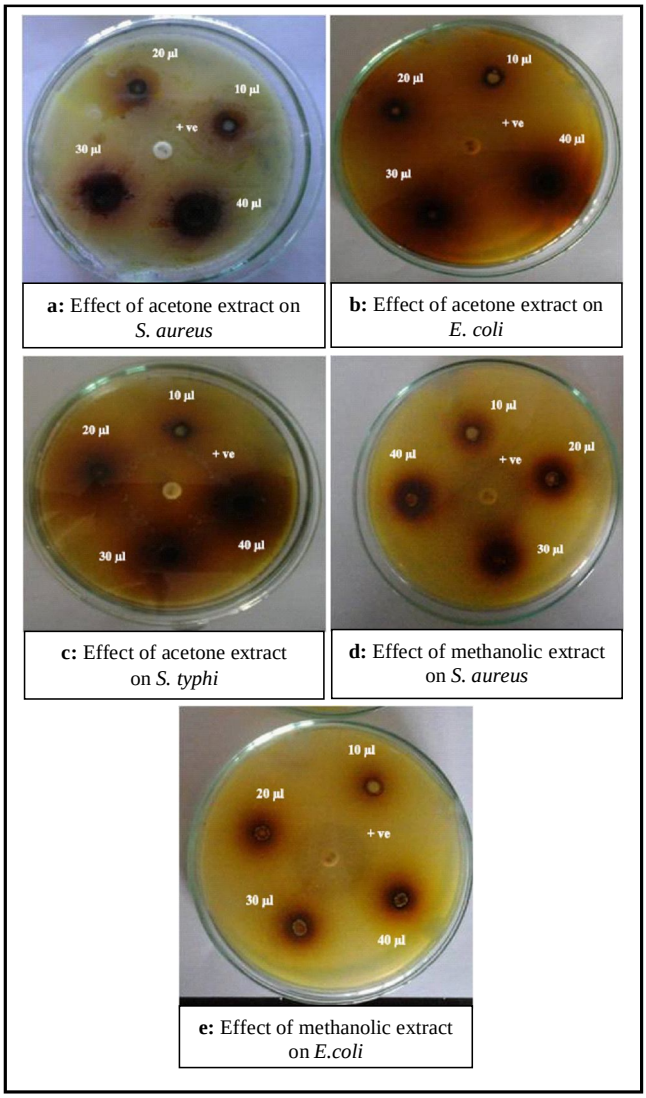


Figure 7: Effect of *J. regia* bark extract on pathogenic bacteria.

3.6 MIC of aqueous extract of *A. racemosus* and acetone extract of *J. regia*

The MIC is the minimum concentration required to inhibit or kill the pathogenic microorganism. It was carried out using aqueous extract of *A. racemosus* and acetone extract of *J. regia* as these extracts were more effective among the others. MIC of selective microbes was carried out because these microbes were found to be more sensitive towards the respective plant extracts.

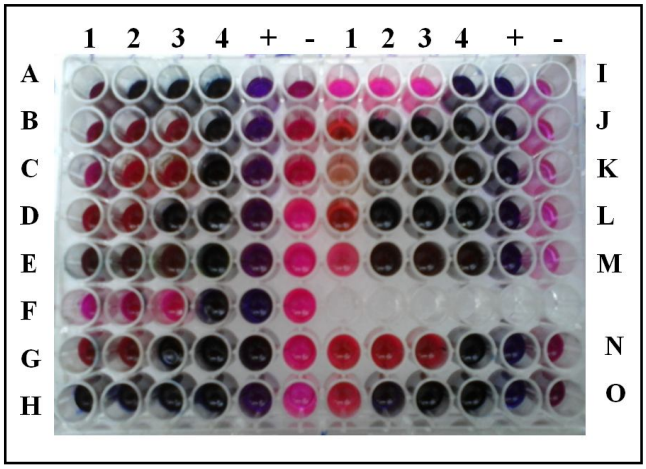


Figure 8: MIC of extracts of *A. racemosus* and *J. regia* on pathogenic isolates.

Table 2: MIC of aqueous extract of *A. racemosus* and acetone extract of *J. regia*

Plant extract (mg/100ul)	Microorganisms				
	Aqueous extract of *A. racemosus*			Acetone extract of *J. regia*	
	S. pneumoniae	*E. coli*	*S. typhi*	*S. aureus*	*S. typhi*
0.8	-	-	-	-	-
0.6	-	-	-	+	-
0.4	+	-	-	+	+
0.2	+	+	+	+	+

– = no bacterial growth ; + = bacterial growth

The MIC of aqueous extract of *A. racemosus* required to inhibit the growth of *E. coli* and *S. typhi* was similar, *i.e.*, 0.4 mg/100 µl whereas in case of *S. pneumonia*, the minimum concentration of 0.6 mg/ml was required. The minimum concentration of acetone extract of *J. regia* required to inhibit the growth of *S. aureus* and *S. typhi* were 0.8 mg/100 µl and 0.6 mg/100 µl, respectively.

4. Discussion

Antimicrobial activity of *A. racemosus* and *J. regia* plant extracts was checked against different clinical isolates (*E. coli*, *K. pneumonia*, *S. typhi*, *S. aureus*, *S. pneumoniae* and *P. aeruginosa*). Good antibacterial activity of petroleum ether, chloroform, acetone, methanol and aqueous extracts of *A. racemosus* and *J. regia* was observed against different pathogenic bacterial strains. Among different solvents, aqueous extract of *A. racemosus* and acetone extract of *J. regia* were found to be most effective. In the present study, maximum inhibitory effect of aqueous extract of *A. racemosus* was found against *S. typhi* and *E. coli* (18 mm at 4 mg/ml) by agar well diffusion method. Chloroform, petroleum ether and methanol

extracts of *A. racemosus* also showed good results against *E. coli, S. typhi, S. aureus* and *P. aeruginosa. A. racemosus* showed activity against both Gram-positive and Gram-negative bacteria. Battu and Kumar (2010) evaluated the antimicrobial potential of *A. racemosus* extract against *B. pumilis*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *S. aureus*, *A. niger* and *C. albicans*. The maximum zone of inhibition of 14.3 ± 0.2 mm was reported earlier against Gram-positive *S. aureus*; however, it was 14.0 mm against Gram-negative *E. coli*, and 16.2 ± 0.2 mm against *C. albicans* which are comparable to the results obtained during the present study. The study conducted by Sinha and Biswas (2011) revealed that chloroform extract exhibited significant activity against *Bacillus subtilis* and moderate activity against *S. aureus* and *S. dysenteriae.*

It has also been reported that minimum concentration of 0.4 mg/ 100 µl of aqueous extract of *A. racemosus* was required to inhibit the growth of *P. aeruginosa*. Earlier, ethanol and chloroform extract showed MIC of 12.5 and 25 mg/ml against *B. pumilis*, *S. aureus*, *E. coli* and *C. albicans*, respectively (Battu and Kumar, 2010). Methanolic extract of *J. regia* showed good inhibitory effect against *S. pneumoniae* (13 mm, 4 mg/ml) and *S. aureus* (22 mm, 4 mg/ml). Acetone extract also showed good activity against *S. aureus* (24 mm, 4 mg/ml).

The minimum concentration of 0.6 mg/100 µl of acetone extract of *J. regia* was required to inhibit the growth of *S. aureus.* Darmani *et al*. (2006) have also reported the inhibition of growth of various cariogenic bacteria (*S. mutans, S. salivarius, Lactobacillus casei,* and *Actinomyces viscosus)* by walnut aqueous extract. Allaie *et al.* (2018) observed the activity of *J. regia* against various pathogenic isolates such as *E. coli* (22 mm), *P. aeruginosa* (12 mm), *K. pneumonia* (16 mm), *P. mirabilis* (17 mm), and minimum activity was observed against *S. saprophyticus* (07 mm). The results were comparative with the present study. Earlier reports have also showed that walnut leaves could be used as an easily available source of natural compounds to inhibit the growth of different Gram-positive bacteria, responsible for dental plaques and oral hygiene problems (Sharafati *et al*., 2011). Acetone extract of *J. regia* has been reported to be more effective as an antimicrobial agent against pathogenic bacteria (Deshpande *et al*., 2011).

5. Conclusion

From the present investigation, it was found that since both root extract of *A. racemosus* and bark extract of *J. regia* could inhibit growth of different pathogenic bacterial strains, hence these can be effectively used for curing the diseases caused by the bacteria tested. Among different solvent extracts, aqueous of *A. racemosus* and acetone of *J. regia* were found most effective. The demonstration of inhibitory activity against both Gram-positive and Gram-negative bacteria is an indication of broad spectrum activity of these plant extracts which can be explored for drug development. The effectiveness of traditional formulations against various pathogenic strains can be enhanced by technical inputs which will not only help in the validation of treatment methods, used by local herbal practitioners but will also increase the effectiveness of phytochemicals. Further, studies are also required to fully understand the toxicity and the molecular mechanisms involved with the antimicrobial activity of these extracts. Efforts are also needed to purify and characterize the bioactive compounds to enhance the antimicrobial potential of the crude plant extracts. For this, advanced techniques like nanotechnology, super critical fluid extraction (SCFE) and analysis through gas chromatography (GC), mass spectroscopy (MS) and NMR spectroscopy can be used.

Conflict of interest

We declare that we have no conflict of interest.

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