Applied Biological Research 15 (2): 149-153; (2013) **Short Communication**

ANTIMICROBIAL AND PHYTOCHEMICAL SCREENING OF RHIZOME EXTRACTS OF SOME NATIVE MEDICINAL PLANT OF HIMACHAL PRADESH (INDIA)

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ABSTRACT

The ethanolic and aqueous extracts of rhizomes of five medicinal plants (*Agave sisaliana, Euphorbia royleana, Zantedeschia aetthiopica, Stephania glabra* and *Mirabilis jalapa*), collected from Solan district of Himachal Pradesh (India), were assayed for antimicrobial activity against eight bacteria including some human pathogens. The ethanolic and aqueous extracts of these plants were less effective against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* whereas *M. jalapa* extract was ineffective against *Shigella* sp. The qualitative phytochemical analysis confirmed the presence of various phytochemicals like tannins, alkaloids, saponins, steroids, phenols and glycosides in the rhizome extracts of these plants. The plant extracts tested possessed antimicrobial potential. Further, the presence of phytochemicals such as tannins, alkaloids, saponins, etc. in the rhizome extracts of these plants suggest their anticancer potential.

Key words: Antimicrobial activity aqueous extract, ethanolic extract, phytochemical, pathogens, rhizome

INTRODUCTION

Herbal medicines, based on their traditional uses in the form of powder, liquid or mixtures, have frequently been used as treatment against various ailments in India. These herbal plants have proved pivotal in the development of new drugs or phytomedicines for treatment of diseases. Several plant species (medicinal or non-medicinal) have been used for centuries as remedy against human diseases as these contain components of therapeutic value (Iwu *et al.*, 1999). Recent acceptance of traditional medicine as alternative form of health care and the development of microbial resistance to the available antibiotics has lead the researchers to explore antimicrobial properties of medicinal plants. The use of various plant parts such as leaves, roots, shoot, rhizomes, etc. or their extracts as complementary and alternative medicine has dramatically increased during last 20 years (Rios and Recio, 2005). Antimicrobial potential of different medicinal plants is extensively studied throughout the world (Kaur and Arora, 2009). The plant rhizomes and tuberous roots have extensively been used in indigenous systems of medicines in Asia especially in India, Pakistan and China for the treatment of diseases like cancer, jaundice and asthma (Asif, 2012).

The rhizomes are the horizontal underground plant stem capable of producing the shoot and root systems of a new plant. This capability allows the parent plant to propagate vegetatively and enables a plant to perennate underground. The rhizome family includes ginger, turmeric and galangal among a

few other, lesser known rhizomes. Ginger is a tropical Asian herb which possesses potential antimicrobial properties and is often used against cough and cold disease (Auta *et al.*, 2011).

The therapeutic value of various plant parts of *Agave sisaliana* (Sisal), *Euphorbia royleana* (Danda thor), *Zantedeschia aetthiopica* (Arum lily), *Stephania glabra* (Biskhapra) and *Mirabilis jalapa* (Lal gulabas) have been reported by different investigators (Muthumani *et al.*, 2009; Ravishankar and Sreeramulu, 2009). These plants are wildly grown in the hills of Himachal Pradesh (India). However, there is no report about the antimicrobial potential of rhizome extracts (both aqueous and alcoholic) from the above plants. The present study was, therefore, aimed to assess the antimicrobial potential of aqueous and organic rhizome extracts from these plants against some known clinical pathogenic bacteria. Also, phytochemical screening was carried out to identify the major biologically active phyto-constituents in the extracts.

MATERIALS AND METHODS

All the chemicals and standard antibiotics used in present study were procured from Hi-Media, Mumbai, India and all the solvents used were of analytical grade. Five different rhizome plants i.e. *Agave sisaliana* (Sisal), *Euphorbia royleana* (Danda thor), *Zantedeschia aetthiopica* (Arum lily), *Stephania glabra* (Biskhapra) and *Mirabilis jalapa* (Lal gulabas) were collected from Solan. Himachal Pradesh (India) and their identity got authenticated at the Department of Botany, SILB, Solan, India. The plants were dried, external covering of rhizomes removed and main rhizomes (10 g) crushed to prepare liquid extracts in liquid nitrogen. Fresh clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Serratia marcesens, Shigella* sp. and *Salmonella typhi* were procured from the Department of Microbiology, SILB, Solan, India. The cultures were maintained on nutrient agar slants, sub-cultured regularly and stored at 4°C as well as at -80°C as glycerol preserve.

For the preparation of plant extracts, rhizomes of test plants were first cleaned, then washed with tap water followed by distilled water and the extracts of rhizome obtained in water (aqueous extract) and ethanol (ethanolic extract) as per the method of Sameera *et al*. (2010) with slight modifications. For aqueous extract, 100 g rhizomes of each test plant (*A. sisala, E. royleana, Z. aethiopica, S. galabra* and *M. jalapa*) was cut into small pieces, crushed in a pestle and mortar along with 100 mL sterile distilled water and ground to paste. The mixture was allowed to stand overnight (24 h) in a shaking water bath maintained at 37°C. The supernatant was filtered through a muslin cloth and then through Whatman filter paper No. 1. The supernatants were collected in ampoules and different concentration (10, 20, 30, 40 and 50 mg mL^{-1}) of extracts prepared for bioassay. For ethanolic extract, 50 g air-dried rhizomes of test plants were ground to fine powder in a pestle and mortar in liquid nitrogen. Powered samples were soaked in 100 mL 95% ethanol for 72 h. The supernatant was decanted in a 100 mL conical flask and kept in refrigerator till further use.

The antimicrobial activity was tested in triplicate reaction by using disc-diffusion and well-diffusion method of Bauer *et al.* (1966). The phytochemical analysis of rhizome extracts for the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpinoids and alkolids was performed as per the methods of Sofowora (1993) and Harborne (1998). In the present study only antagonistic potential of different plant extracts was studied so only simple CRD stat was applied.

RESULTS AND DISCUSSION

The ethanolic and aqueous extracts of *Stephania galabra* produced maximum inhibition zones of 5.2, 5.6, 5.9 and 6.1 mm; and 5.4, 5.5, 5.7 and 5.9 mm, respectively, against *Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa* and *Serratia marcesens*, respectively (Table 1). A slightly low inhibitory effect was observed against *Escherichia coli* (3.1 and 3.0 mm), *Bacillus subtilis* (3.0 and 3.2 mm) and *Staphylococcus aureus* (1.9 and 1.7 mm). The ethanolic and aqueous extract of *S.*

Table 1: Antibacterial activity of various plant extracts against test organisms

Plant extracts / Bacteria	Zone of inhibition (mm) of aqueous and ethanolic extract (10 mg mL^{-1})									
	A. sisalana		*E. royeana*		*Z. aethiopica*		*S. galabra*		*M. jalapa*	
	Et*	Aq*	Et	Aq	Et	Aq	Et	Aq	Et	Aq
Staphylococcus aureus	0.00	3.50	3.13	3.00	2.30	2.63	1.90	1.73	2.53	2.00
Klebsiella pneumonia	2.50	2.53	3.23	2.93	3.93	3.70	5.23	5.40	2.73	2.23
Bacillus subtilis	0.00	3.20	3.20	3.23	2.53	2.00	3.03	3.23	2.30	2.10
Serratia marcesens	0.00	0.00	0.00	0.00	0.00	0.00	6.10	5.90	3.83	4.00
Escherichia coli	4.23	3.53	3.73	3.50	2.90	2.73	3.13	3.03	3.73	3.23
Pseudomonas aeruginosa	0.00	3.20	3.40	3.50	4.13	4.00	5.93	5.70	3.53	3.80
Salmonella typhi	2.83	3.53	3.13	3.03	3.53	3.53	5.60	5.53	4.23	3.73
Shigella sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$CD_{0.05}$	0.13	0.16	0.19	0.16	0.19	0.16	0.19	0.19	0.23	0.18

*Et = ethanolic extract; Aq = aqueous extract

galabra was ineffective against *Shigella* sp. Ethanolic and aqueous extracts of *Mirabilis jalapa* showed slightly less inhibitory effect on *Klebsiella* sp., *S. typhi, P. aeruginosa* and *S. marcesens with* zone of inhibitions as 2.7, 4.2, 3.5 and 3.8; and 2.2, 3.7, 3.8 and 4.0 mm, respectively. Inhibitory effect was less in case of *E. coli, B. subtitlis* and *S. aureus.* Ethanol and aqueous extracts of *M. jalapa* were ineffective against *Shigella* sp. Antibacterial activity of ethanolic and aqueous extracts of *Agave sisalana*, *Euphorbia royleana* and *Zantedeschia aetthiopica* was less effective against the test indicators as compared to *S. galabra* and *M. jalapa* (Table 1). The ethanolic extract of *S. galabra* was most effective among all the plant extracts used in present study, as it gave highest zone of inhibition against *S. marcesens* (Table 1).

Bioactive ingredient such as tannins, alkaloids, saponins, steroids, phenols and glycosides were detected in rhizome of *Z. aethiopica* and *M. jalapa* (Table 2). Alkaloids were detected in all the plant extracts. Phytochemical screening of ethanolic extracts of *A. sisalana, E. royeana, Z. aethiopica, S. galabra and M. jalapa* showed the presence of glycosides, saponins, tannins, alkaloids, steroids, glycosoids, soluble starch and terpenoids. According to Kennedy and Wightman (2011), the presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. Hence, the presence of secondary metabolites in *A. sisalana, E. royeana, Z. aethiopica, S. galabra* and *M. jalapa* can be responsible for their potential as drugs. The inhibitory activity exhibited by the secondary metabolites is in agreement with Paiva *et al.* (2010) who linked the antibacterial properties of plants to the presence of secondary metabolites. Several plants rich in alkaloids possess anti-malarial activity against a number of microorganisms. For example,

Table 2: Phytochemical screening of different plant rhizome extracts

Phytochemical	*A. sisalana*	*E. royleana*	*Z. aethiopica*	*S. galabra*	*M. jalapa*
Alkaloid	+	+	+	+	+
Tannin	-	-	-	-	+
Anthraquinone	-	-	+	-	-
Glycoside	-	-	+	-	+
Steroid	-	-	+	-	-
Terpenoid	-	-	+	+	+
Saponin	+	-	+	+	+
Flavonoid	-	-	-	-	+
Soluble starch	-	-	+	-	+

+ = present; - = absent

Adebayo *et al.* (1983) investigated the antimicrobial activity of leaf extract of *Eugenia uniflora* and reported that alkaloids, tannins and glycosides were detected and that the ethyl acetate and methanolic leaf extracts of the plant were active against *E. coli, P. vulgaris, K. pneumoniae* and *A. niger*. The zone of inhibition of the tested organisms indicated their susceptibility to the plant parts used in this study. In the present study, the zone of inhibition varied from one organism to another and from one plant parts to another at different concentrations. The ethanolic and aqueous extracts of *A. sisalana, E. royeana, Z. aethiopica, S. galabra* and *M. jalapa* exhibited antimicrobial activities against the test organisms. It may, therefore, be suggested that the rhizomic extracts of these plants can be used in chemotherapy. This study is the preliminary report on antimicrobial activity of aqueous and ethanolic extracts of *A. sisalana, E. royeana, Z. aethiopica, S. galabra* and *M. jalapa* against these human pathogenic strains. Several reports describe the antibacterial and antifungal properties of different herbs and spices (Gull *et al.,* 2012); however, the information about the exact mechanism of their antimicrobial action is meager (Gur *et al.,* 2006; Yusha'u *et al*., 2008; Oskay *et al.,* 2009; Belguith *et al.,* 2010; Poeloengan *et al*., 2011).

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