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A STUDY OF ANTIBACTERIAL ACTIVITY OF *ANDROGRAPHIS PANICULATA* LEAF AND STEM BARK EXTRACTS AGAINST SOME CLINICAL PATHOGENIC BACTERIA'S

Jyoti Pandey*[1], Vimal K Saini[1], Shashi Tiwari[2], Wasim Raja[3]

[1*]Shri Gurunanak Mahavidyalya, Jabalpur, Madhya Pradesh, India.
[2]Government Autonomous M.H. College of Home Science and Science for Women, Jabalpur, Madhya Pradesh, India.
[3]Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh, India.

ABSTRACT

The use of plant extracts for antimicrobial activity and other diseases have been observed to be promising remedies since ancient time in Chinese medicine, Ayurveda, Arabic, and Unani medicine. The plants have traditionally furnish a source of hope for novel drug compounds, as plant herbal mixtures have made large endowment to human health and well-being. The use of plant extracts with known antimicrobial properties can be of appreciable significance for therapeutic treatment. Presently, the research has been initiated to study the antibacterial activity of chloroform, and methanol extracts of *Andrographis paniculata* to emphasize the potential of herbal components in the field of medical science to kill various dreadful pathogens. The agar well diffusion method was followed to evaluate the antibacterial activity of leaf and stem extracts of *A. paniculata* against *Bacillus subtilis, S. aureus, Enterococcus, P. aeruginosa, K. pneumonia and E. coli.* The result revealed that all the doses of both extracts of *A. paniculata* potentially inhibited the growth of all the pathogens tested. Hence, the present investigation evaluates the potential anti-bacterial activity of methanol extract of leaf and stem extract of *A. paniculata.*

KEYWORDS

Andrographis paniculata, Antimicrobial activity, Pathogens, Methanol and Leaf and Stem bark extract.

Author for Correspondence:
Jyoti Pandey,
Shri Gurunanak Mahavidyalya,
Jabalpur, Madhya Pradesh, India.

Email: drwasimraja84@gmail.com

INTRODUCTON

Plants produce a wide range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs used today are acquired from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine (Sukanya *et al*, 2009)[1]. Medicinal plants are finding their way into

pharmaceuticals, cosmetics, and nutraceuticals. In pharmaceutical field medicinal plants are largely used for the broad range of substances present in plants which have been used to treat infectious as well as chronic diseases (Okigbo *et al*, 2009)[2]. The drugs already in use to treat infectious disease are of concern because drug safety remains a huge global issue. Almost all of the synthetic drugs cause side effects and also most of the microbes developed resistant against the synthetic drugs. To alleviate this problem, antimicrobial compounds from potential plants should be explored. These drugs from plants are fewer side effects, less toxic, scanty and also cost effective. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Kadhim *et al*, 2016)[3]. Treatment with medicinal plants having antibacterial activity is potentially beneficial alternative and promising source of pharmaceutical agents (Sridevi *et al*, 2010)[4]. Plants are rich in a wide variety of secondary metabolites of phytochemical constituents such as tannins, alkaloids and flavonoids, which act against different diseases (Govind and Madhuri, 2010)[5]. In addition, plant derived phytomedicines provide a cheaper source for treatment and significant accuracy than chemotherapeutic agents (Punitha *et al*, 2008)[6]. *Andrographis paniculata,* commonly known as 'King of Bitter, is a small, annual, branched and erect plant belongs to the family Acanthaceae. It grows abundantly in south eastern Asia including India, Sri Lanka, Java, Pakistan, Indonesia and Malaysia. It prefers to grow well in a diversity of habitats such as moist, shady areas, hill slopes, plains, farms, seashores, waste lands and dry or wet lands (Prajapati, 2003)[7]. It is rich in a wide variety of phytochemical constituents such as diterpenes, flavonoids and lactones (Chang *et al*, 1987)[8]. *A. paniculata* is extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases in Indian traditional system as well as in tribal medicine applications. The therapeutic value of Kalmeg is due to its mechanism of action by enzyme induction.

It is a powerful cold property herb, used in fevers and to dispel toxins from the body. It is used to treat gastrointestinal tract and upper respiratory infections, fever, herbs, sore throat, hepatitis and a variety of other chronic and infectious diseases (Chopra, 1956)[9]. The herbs and its isolates like, isoandrographolide, neoandrographolide, andrographolide, isoandrographolide are reported to possess anti-inflammatory activity (Liu, 2007)[10] hepatoprotective (Shukla, 1992)[11], anti - diabetic (Umamaheswari *et al*, 2007)[12], anti - malarial (Misra *et al*, 1992)[13], anti - microbial (Singha, 2003)[14], piles and gonorrhea (Prajapati, 2003)[7]. Herbal drugs in disease management are attain success, because they are cost effective, eco-friendly and have minimal side effects (Ahilan *et al*, 2010)[15]. Hence, this work made an attempt to study the antimicrobial activity of *Andrographis paniculata* against various pathogenic microorganisms.

MATERIAL AND METHODS

Collection of *A. paniculata*

The experimental plant species, *A. paniculata* was purchased from the local herbal market. The plant was authenticated and the voucher specimen was deposited in the herbarium of Shri Gurunanak Mahavidyalya, Jabalpur, Madhya Pradesh, India.

Preparation of plant powder

Fresh *A. paniculata* plants were washed thoroughly in tap water followed by distilled water and were then shade dried until all the water content was lost completely. Dried plants were crushed and powdered using blender. Fine powder was obtained after sieving and stored in airtight container until further use.

Preparation of experimental plant extracts

The plant powder was extracted with methanol solvent with an increasing polarity. The successive extraction was done by a cold maceration process for seven days with regular agitation. After seven days of cold maceration process it was filtered through sterile muslin cloth and the solvent was evaporated using soxhlet apparatus. The residues obtained after evaporation were stored at -20ºC until used for experimentation.

Test microorganisms

To evaluate the antimicrobial activity of *A. paniculata* extracts, six species/ strains of microorganisms were selected, namely *Bacillus*

subtilis, S. aureus, Enterococcus, P. aeruginosa, K. pneumonia and *E. coli*. All these bacterial strains were collected from clinical lab and sub cultured in nutrient agar medium and used for antimicrobial susceptibility test.

Antibacterial assay

The potential antibacterial activity of *A. paniculata* extract was studied through agar well diffusion method (Murry *et al*, 1995)[16]. The sterile petri dishes were filled with 25ml of agar and allowed the agar to get solidified. Prior to streaking the plates with bacterial culture, 5mm diameter wells were punched in the medium using a sterile borer. After the agar gets solidified the bacterial cultures were inoculated by spreading in the petri plates using sterile cotton swabs. Then 0.1ml of plant extract in peptone water was directly applied to the well made on the surface of agar containing bacterial lawn. Positive control was maintained with different antibiotics and wells containing solvent alone was maintained as negative control. The inoculated plates were incubated overnight at 37ºC for allowing the bacterial growth and the diameter of zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

In the present investigation, methanol leaf and stem bark extract of *A. paniculata* were studied against *Bacillus subtilis, S. aureus, Enterococcus, P. aeruginosa, K. pneumonia and E. coli* using agar well diffusion method. The results showed that the extract of *A. paniculata* has a concentration dependent antibacterial activity with more sensitivity for Gram negative bacteria than Gram positive bacteria used in the study. The extracts of *A. paniculata* showed considerable antibacterial activity at all the four concentrations 100, 75, 50 and 25mg/ml (Table No.1 and 2). Table No.3 shows the sensitivity of the tested bacteria to the standard antibiotics. Examination of this study clearly revealed that methanol extracts of *A. paniculata* act as a significant growth inhibitor against broad spectrum of pathogens and act as a potent antimicrobial activator.

Medicinal plants are the prime sources of new medicines and may constitute an alternative to the usual drugs. Medicinal and aromatic plants are used on a wide scale in medicine against drug resistant bacteria (Tepe *et al*, 2004)[17]. In this study all the *A. paniculata* extracts exhibited varying degree of inhibitory activity against the growth of all the microorganisms tested except Pseudomonas aeruginosa. This result was supported by many of the researchers who already reported that *A. paniculata* as potent antimicrobial activator. (Mishra *et al*, 2013)[18] reported that 75% methanol extract of *A. paniculata* leaves was found to be active against *S.aureus, E. faecalis* and *M. tuberculosis.* (Zaidan *et al*, 2005)[19] have reported that the water extracts of *A. paniculata* possess a potential antibacterial activity towards both gram positive and gram negative bacteria. According to the results of (Humnabadkar and Kareppa 2012)[20], the aqueous extracts of *A. paniculata* showed maximum antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* (Hosamani *et al*, 2011)[21], have reported that the acetone and alcohol extracts of *A. paniculata* with higher inhibitory activity against *Bacillus subtilis* and *Staphylococcus aureus*. Research conducted on other plants also showed positive result on antimicrobial activity. (Turker *et al*, 2009)[22], examined the aqueous and alcoholic extracts of *Nupharlutea, Nymphaea alba, Stachysannua, Genistalydia, Vinca minor, Fragaria herbs* of Turky with antibacterial activity against A. hydrophila, Enterococcus faecalis, Lactococcusgarvieae, Streptococcus agalactiae and Yersinia ruckeri bacteria isolated from fish. (Mahesh and Satish 2008)[23].

In our present investigation, methanol leaf and stem bark extract of A. paniculata were studied against *Bacillus subtilis, S. aureus, Enterococcus, P. aeruginosa, K. pneumonia and E. coli* using agar well diffusion method. The results showed that the extract of *A. paniculata* has a concentration dependent antibacterial activity with more sensitivity for Gram negative bacteria than Gram positive bacteria used in the study. The extracts of *A. paniculata* showed considerable antibacterial activity at all the four concentrations 100, 75, 50 and 25mg/ml (Table No.1 and 2). Table No.3 shows the sensitivity of the tested bacteria to the standard antibiotics. Examination of this study clearly revealed that methanol extracts of *A. paniculata* act as a significant growth inhibitor against broad

spectrum of pathogens and act as a potent antimicrobial activator.

Generally gram positive bacteria were more sensitive to plant extracts because of the presence of a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts (Tajkarimi *et al*, 2010)[24]. The resistance of the gram negative bacteria could be attributed to its cell wall structure. Gram negative bacteria have a powerful permeability barrier, composed of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding plant extract.

It has been discussed earlier that gram negative bacteria are usually more resistant to the plant originated antimicrobials and even show no effect, compared to gram positive bacteria (Stefanello, 2008)[25].

Results obtained from this study, indicated that, the plant extracts showed the strongest antimicrobial activity than the control. Further studies are needed for these potent plant extracts to evaluate the other parameters of antimicrobial activity (e.g., toxicity, *in vivo* efficacy, antiviral and antiparasitic and antimycobacterial activity).

Table No.1: The study of anti-bacterial activities of *Andrographis paniculata leaf* extracts using disk diffusion Method (Mean± SE)

S.No	Name of Bacteria	Zone of Inhibition (In MM)			
1	Gram Positive (+)	**100%**	**75%**	**50%**	**25%**
	Bacillus subtilis	3.66 ± 0.78	8.33 ± 0.94	8.66 ± 1.05	6.33 ± 0.64
	S. aureus	4.33 ± 0.84	8.33 ± 0.65	7.33± 0.66	8.33 ± 0.74
	Enterococcus	5.66 ± 0.97	8.33 ± 0.74	8.33 ± 0.94	10.33±0.74
2	Gram Negative (-)				
	P. aeruginosa	4.66 ± 0.87	9.66 ± 1.05	11.33± 0.94	7.33± 0.66
	K. pneumoniae	6.33 ± 1.02	8.66 ± 0.74	9.33 ± 1.05	10.66±0.74
	E. Coli	5.33 ± 0.94	8.66 ± 0.66	10.33±0.74	9.33 ± 1.10

Table No.2: The studies of anti-bacterial activities of *Andrographis paniculata stem bark* extract using disk diffusion Method (Mean± SE)

S.No	Name of Bacteria	Zone of Inhibition (In MM)			
1	Gram Positive (+)	**100%**	**75%**	**50%**	**25%**
	Bacillus subtilis	5.33 ± 0.97	7.33± 0.94	5.66 ± 0.97	5.33 ± 0.94
	S. aureus	6.66 ± 0.74	8.66 ± 1.20	8.33 ± 0.66	8.33 ± 0.46
	Enterococcus	9.33 ± 0.74	8.33 ± 0.74	8.66 ± 1.20	7.66 ± 0.87
2	Gram Negative (-)				
	P. Aeruginosa	8.00 ± 0.57	6.33 ± 0.74	6.33 ± 0.46	3.66 ± 0.78
	K. Pneumoniae	9.33 ± 0.46	8.33 ± 0.94	8.66 ± 1.05	8.66 ± 0.66
	E. Coli	7.66 ± 0.46	7.66 ± 0.87	8.66 ± 1.04	5.33 ± 0.97

Table No.3: The study of anti-bacterial activities of standard antibiotics using disk diffusion method

S.No	Name of Bacteria	Zone of Inhibition (In MM)			
1.	Gram Positive (+)	**NX10**	**OF5**	**E15**	**GEN10**
	Bacillus subtilis	25	29	22	20
	S. aureus	30	25	10	23
	Enterococcus	34	28	13	25
2.	Gram Negative (-)				
	P. Aeruginosa	35	32	15	30
	K. Pneumoniae	30	25	10	18
	E. Coli	31	30	15	32

CONCLUSION

A. paniculata has been used in Ayurveda, Unani and Siddha systems of medicine from ancient times. It has wide spectrum of pharmacological activities either in the form of powder, extracts or in its isolated compounds with minimum side effects; several products fortified with extract or isolated compounds have been launched in national and international markets for various diseases. In our present investigation, methanol leaf and stem bark extract of *A. paniculata* were studied against *Bacillus subtilis, S. aureus, Enterococcus, P. aeruginosa, K. pneumonia and E. coli* using agar well diffusion method. The results showed that the extract of *A. paniculata* has a concentration dependent antibacterial activity with more sensitivity for Gram negative bacteria than Gram positive bacteria used in the study. The extracts of *A. paniculata* showed considerable antibacterial activity at all the four concentrations 100, 75, 50 and 25mg/ml (Table No.1 and 2). Table No.3 shows the sensitivity of the tested bacteria to the standard antibiotics. Examination of this study clearly revealed that methanol extracts of *A. paniculata* act as a significant growth inhibitor against broad spectrum of pathogens and act as a potent antimicrobial activator. The enriched *A. paniculata* plant extracts are further studied for their antibacterial properties against selective human pathogens. In this context, the present study was carried out to find out the antibacterial potential of various solvent based extract of *A. paniculata* against selective human pathogens. The antibacterial activity of *A. paniculata* may be due to the presence of active principle called andrographoloid. In future, the improvements of active principle andrographoloid content in A. paniculata using plant growth promoting rhizobacteria (PGPR) are studied. The enriched A. paniculata plant extracts are further studied for their antibacterial properties against selective human pathogens.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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