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Antibacterial activity of *Andrographis paniculata* extracts

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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 chloroform and methanol extracts of *A. paniculata* against *Escherichia coli, Aeromonas hydrophila, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Klebsiella pneumonia* and *Salmonella typhi*. The result revealed that all the doses of chloroform and methanol extracts of *A. paniculata* potentially inhibited (10 - 16mm in diameter) the growth of all the pathogens tested except *Pseudomonas aeruginosa*. Hence, the present investigation evaluates the potential anti bacterial activity of chloroform and methanol extracts of *A. paniculata*.

Keywords: *Andrographis paniculata,* antimicrobial activity, pathogens, chloroform, methanol

1. Introduction
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 [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 [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 [3].

Treatment with medicinal plants having antibacterial activity is potentially beneficial alternative and promising source of pharmaceutical agents [4, 5]. Plants are rich in a wide variety of secondary metabolites of phytochemical constituents such as tannins, alkaloids and flavonoids, which act against different diseases [6, 7, 8]. In addition, plant derived phytomedicines provide a cheaper source for treatment and significant accuracy than chemotherapeutic agents [9].

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 [10]. It is rich in a wide variety of phytochemical constituents such as diterpenes, flavonoids and lactones [11].

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 [12]. The herbs and its isolates like, isoandrographolide, neoandrographolide, andrographolide, isoandrographolide are reported to possess anti-inflammatory activity [13, 14], hepatoprotective [15], anti - diabetic [16, 17], anti - malarial [18], anti - microbial [19], anti –HIV activity [20]. Immunostimulatory activity [21], anti - cancer [22], and helps in arresting dysentery, cholera, influenza, bronchitis, swellings and ithches, piles and gonorrhea [10]. Herbal drugs in disease management are attain success, because they are cost effective, eco-friendly and have minimal side effects [23]. Hence, this work made an attempt to study the antimicrobial activity of *Andrographis paniculata* against various pathogenic microorganisms.

2. Materials and Methods

2.1 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 (Specimen No. JACZOO IM1) was deposited in the herbarium of PG & Research Centre of Zoology, Jayaraj Annapackiam College for Women (A), Periyakulam. S. India.

2.2 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.

2.3 Preparation of experimental plant extracts

The plant powder was extracted with three different solvents with an increasing polarity (chloroform and methanol solution). The successive extraction was done by a cold maceration process for seven days with regular agitation [24, 25]. 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 -20°C until used for experimentation.

2.5 Test microorganisms

To evaluate the antimicrobial activity of *A. paniculata* extracts, nine species/ strains of microorganisms were selected, namely *Klebsiella pneumonia, Bacillus subtilis, Aeromonas hydrophila, Proteus vulgaris, Salmonella typhi,, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa.* All these bacterial strains were collected from clinical lab and sub cultured in nutrient agar medium and used for antimicrobial susceptibility test.

2.6 Antibacterial assay

The potential antibacterial activity of *A. paniculata* extract was studied through agar well diffusion method [26]. The sterile petri dishes were filled with 25ml of Muller Hinton 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 Muller Hinton agar containing bacterial lawn. Positive control was maintained with antibiotic amikacin (3mg) and wells containing solvent alone was maintained as negative control. The inoculated plates were incubated overnight at 37°C for allowing the bacterial growth and the diameter of zone of inhibition was measured in mm.

3. Results

3.1 Anti bacterial activity

In the present investigation, chloroform and methanol extracts obtained from *A. paniculata* were studied against *K. pneumonia, B.subtilis, A. hydrophila, P. vulgaris, S. typhi, S.aureus, S. pyogenes, E.coli* and *P. aeruginosa* using agar well diffusion method. As per the results shown in table 1, effective antibacterial activity was observed in mid (150 mg) and higher doses (200 mg) of chloroform and all the doses of methanol extracts of *A. paniculata* against *Klebsiella pneumonia* and *Bacillus subtilis* with the zone of inhibition ranging from 10-16 mm (Table 1).The higher dose (200 mg) of methanol extract inhibited the growth of *Aeromonas hydrophila* and *Proteus vulgaris* (Zone of inhibition - 12 mm). The growth of *Salmonella typhi* was inhibited by mid (150 mg) and higher dose (200 mg) of methanol extract (Zone of inhibition - 12 mm). All the doses of chloroform extract potentially inhibited the growth of *Staphylococcus aureus* with the zone of inhibition ranging from 10 - 16 mm. The higher dose (200 mg) of chloroform extract inhibited the growth of *Streptococcus pyogenes* (Zone of inhibition - 11 mm). The growth of *E.coli* was inhibited by the mid (150 mg) and higher dose (200 mg) of chloroform and methanol extract of *A. paniculata* with the zone of inhibition ranging from 10 - 13 mm. None of the extracts inhibited the growth of *Pseudomonas aeruginosa*. Examination of this study clearly revealed that chloroform and methanol extracts of *A. paniculata* act as a significant growth inhibitor against broad spectrum of pathogens and act as a potent antimicrobial activator.

Table 1: Zone of inhibition of different extracts of *A. paniculata* against different microorganisms (* ND- Not Detected)

S. No	Name of the bacteria	Positive control (Amikacin, 3mg)	Zone of Inhibition (mm)					
			CE (mg)			ME (mg)		
			100	150	200	100	150	200
1	*Klebsiella pneumonia*	17	ND	11	12	11	12	12
2	*Bacillus subtilis*	16	ND	12	16	10	12	14
3	*Aeromonas hydrophila*	17	ND	ND	ND	ND	ND	12
4	*Proteus vulgaris*	16	ND	ND	ND	ND	ND	12
5	*Salmonella typhi*	16	ND	ND	ND	ND	12	12
6	*Staphylococcus aureus*	18	10	16	16	ND	ND	ND
7	*Streptococcus pyogenes*	17	ND	ND	11	ND	ND	ND
8	*Escherichia coli*	17	ND	10	10	ND	12	13
9	*Pseudomonas aeruginosa*	16	ND	ND	ND	ND	ND	ND

4. Discussion

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 [27]. 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) [28] 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) [29] 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) [30], the aqueous extracts of *A. paniculata* showed maximum antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Hosamani *et al.* (2011) [31], 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) [32], examined the aqueous and alcoholic extracts of *Nuphar lutea*, *Nymphaea alba*, *Stachys annua*, *Genista lydia*, *Vinca minor*, *Fragaria* herbs of Turky with antibacterial activity against *A. hydrophila*, *Enterococcus faecalis*, *Lactococcus garvieae*, *Streptococcus agalactiae* and *Yersinia ruckeri* bacteria isolated from fish. Mahesh and Satish (2008) [33], conducted a study on antimicrobial activity of methanol extracts of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritiana* and reported the effective antibacterial activity against *E. coli*, *Pseudomonas fluorescens*, *B. subtilis.*, *Xanthomonas axonopodis Pv malvacearum* and *Staphylococcus aureus*. The methanol extract of *Croton macrostachyus* stem bark induced anti bacterial activity against *K. pneumonia*, *E. coli*, *C. albicans* and *E. aerogenes* with the zone of inhibition between 9.0 ± 1.1 mm and 14.9 ± 1.3 mm [34]. The methanol extract and the ethyl acetate fraction of *Bellis perennis* L flowers exhibited broad spectrum of antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Staphylococcus epidermidis* [35].

The methanol and acetone extracts of *Halimeda micronesia* seaweeds caused maximum inhibitory activity against *A.hydrophila*, *Enterobacter sp*, *Vibrio alginolyticus and Vibrio parahaemolyticus* [36].

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 [37, 38]. The resistance of the gram negative bacteria could be attributed to its cell wall structure. Gram negative bacteria have an 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 [38, 29].

5. Conclusion

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).

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