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In vitro antibacterial activity of *Andrographis paniculata* aqueous, methanolic & ethanolic leaf extracts

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Abstract
Development of antibiotic resistance among most common pathogens has put the light on use of plant and their parts as an alternative therapy option. *Andrographis paniculata* is one of the most used plants as documented by many researchers having antibacterial potential. The present study was done to evaluate its leaves extract efficacy against *Escherichia coli, Staphylococcus aureus*. Aqueous, methanolic and ethanolic leaf extracts were prepared by soxhlet method and used at 500mg/ ml, 750mg/ml and 1000mg/ml concentration in antibacterial test discs (6mm). The extracts were evaluated for antibacterial activity by disc diffusion method in quintuplicate number. No zone of inhibition for could be observed in case of aqueous extracts, while ethanolic extract (@ 1000mg/ml) showed a zone of inhibition (Mean ± SD) of 6.16 ± 0.114 mm & 6.98 ± 0.130 mm against *Escherichia coli, Staphylococcus aureus* with percentages of inhibitions 61.97% and 70.08%. The methanolic extract (@ 750mg/ml and 1000mg/ml) showed zone of inhibition amounting 6.16 ± 0.089, 7.1 ± 0.070 and 7.08 ± 0.083, 7.82 ± 0.130 mm against *Escherichia coli, Staphylococcus aureus* with percentages of inhibitions 61.97%, 71.42% and 71.08%, 78.51% respectively. Methanolic extracts were found to be most effective against both the bacteria.

Keywords: *Andrographis paniculata*, leaf extract, ethanolic, methanolic, disc diffusion, *in-vitro*

Introduction
With the advent of chemical compounds in the health management of human and animals, use of plants and their parts left the scenario of healing (Yineger *et al*., 2007; Khan *et al*., 2019) [30, 13]. The increasing use of chemical compounds or antibiotics has heightened the risk of antibiotic resistance during last decade (Ranabijuli *et al*., 2009; Padhy *et al*., 2016) [21, 18]. Many antibiotic have became resistance to common pathogens (Mohanty *et al*., 2013). This has renewed the interest of treating community for the use of unconventional sources (Yineger *et al*., 2007) [30]. However these unconventional sources and methods are present in the human and livestock health management from distant past and have found its way into the modern scientific era by the act of telling and memorizing.
Use of plants and their parts for treatment and health management of livestock has been advocated by many workers (Jain & Srivastava, 2003; Panda *et al*., 2017) [9, 19]. A wide range of flora is still being used in livestock for curative purpose (Akhtar *et al*., 2000; Garanayak *et al*., 2023) [1, 4].
Andrographis paniculata is one such plant which is still being used for animal health care (Okhuarobo *et al*., 2014; Shahid, 2016; Garanayak *et al*., 2023) [16, 25, 4]. Popularly known as "king of bitters" in English the plant has many pharmacopotentials (Jarukamjorn *et al*., 2010; Okhuarobo *et al*., 2014) [10, 16]. The plant has been reported to possesses antibacterial activities against a wide range of bacteria making it a suitable contender for replacing antibiotics in chemotherapy (Singh *et al*., 2003; Kataky & Handique, 2010; Xu *et al*., 2012; Hossain *et al*., 2021) [26, 12, 29, 8].
The present study was devised to evaluate the antibacterial potential of *Andrographis paniculata* leaves. Different solvents were used to prepare the leaf extracts and their antibacterial property was evaluated using disc diffusion method.

Materials and Methods

The study was conducted at Department of Clinical Medicine, Ethics and Jurisprudence, College of Veterinary Science & Animal Husbandry, Orissa University of Agriculture & Technology, Bhubaneswar, Odisha.

Collection of Plant Materials and processing for extraction

Andrographis paniculata whole plants were collected. Plants having attended maturity or near maturity were specifically selected for the study. The leaves were carefully plucked from the plant, cleaned with water and air dried under shed as described by Padhiari *et al*. (2020) [17]. The leaves were dried up to a period of 35 – 40 days to attend the desired drying effect. All the dried materials were then processed by mechanical grinding and converting the materials to powdered form (Padhiari *et al*., 2020) [17].

Extraction of plant components

For extraction of plant materials procedure described by Padhiari *et al*. (2020) [17] was followed. The detailed procedure is as followed for three types of extracts *viz*. Aqueous, ethanolic and methanolic.

20 gm of dried powdered form of the plant material was weighed. Then the weighed powered sample was placed in thimble chamber of the soxhlet apparatus which is plugged with cotton on the upper side. The extraction solvent (double distil water, 90% methanol and 99.9% ethanol) @ 300 ml was added to the thimble chamber. Care was taken that the side thin glass tube is fill with solvent even after absorption by the sample. Then the condenser was fixed above the extractor. Water channel was ensured for cooling and condensation effect during the procedure.

The extraction solvent was heated at a temperature of 60 – 65 °C in the bottom flask, vaporizes into the sample thimble, condensed in the condenser and drip back. When the liquid content reached the siphon arm, it was emptied into the bottom flask again and the process was continued (Jorgensen & Turnidge, 2007) [11]. To complete one cycle it took around one hour of heating and eight cycles of heating was carried out for complete extraction of the plant materials. The extract was concentrated by evaporation at 70 °C for 8 hours by hot water bath and then dried at room temperature. The concentrated extract was made in Gel form and stored at room temperature for further procedures (Van-Burden & Robinson, 1981; Hanumantappa, 2014) [6, 28]. By the above said method aqueous, methanolic and ethanolic extractions of leaves of *Andrographis paniculata* were prepared.

Antibacterial Disc preparation

The extracts were charged to the discs at different desired concentrations. *Andrographis paniculata* aqueous leaf extract were mixed with distil water @ 500mg/ ml, 750mg/ml and 1000mg/ml along with stirring. After stirring for 1 hr the extract solution was prepared. Sterile antimicrobial discs (6mm diameter) were used for charging the extract solution and preparation of disc with plant extracts. The discs were charged with the extract solution prepared and soaked for 30 minutes. Then the discs were incubated at 37°C for 12 hours. Then the discs were used for antibacterial susceptibility test.

Methanolic and ethanolic extracts of the plant were mixed with 10% DMSO instead of distil water as described above. The methanolic and ethanolic extracts were not completely dissolvable in distil water rending them unable to be absorbed completely in to the discs. Hence 10% DMSO was used for the procedure. The discs prepared were kept in refrigeration till use.

Preparation of bacterial inoculums

Escherichia coli, *Staphyloccocus aureus* bacterial culture were used in the experiment. The pure cultures were obtained from ICMR, Bhubaneswar and were gram stained to ascertain the purity. Standard biochemical and sugar tests were employed for identifying both the bacteria (Harley & Prescott, 2002) [7]. After identification both the bacteria were inoculated in BHI broth and incubated at 37°C over night for getting the pure bacterial culture broths. These broths were diluted in normal saline solution to get inoculums of 10^8 CFU in 0.1ml (Harley & Prescott, 2002) [7]. These inoculums were used for the antibacterial testing purpose.

In – vitro Antibacterial study

The antibacterial study was carried using the plant extract discs prepared at different concentration. The method employed was disc diffusion technique as described by *Borah et al*. (2019) [3]. The agar plates were inoculated with standardized inoculums (10^8 CFU/0.1ml) of the test microorganism spread over the agar surface (Aniel Kumar *et al*., 2010) [2]. Then, filter paper discs (6mm in diameter), containing the test compound at a desired concentration are placed on the agar surface. The petridishes are incubated under suitable conditions (37°C for 24 hours). The diameters of inhibition growth zones are measured. The experiment was done in quintuplicate and the mean diameter of the inhibition zone was calculated. Gentamicin (10 mcg/disc) as standard / positive control and 10% DMSO (Dimethyl sulphoxide) as a negative control were used (Aniel Kumar *et al*., 2010) [2].

Percentage of inhibition

Percentage (%) of inhibition of the test bacterial organism by the test compound was calculated using the given formula;

$$\frac{Mean\ zone\ of\ inhibition\ (test\ compound)}{Mean\ zone\ of\ inhibition\ of\ standard\ (selected\ antibiotic)} \times 100$$

The percentage (%) of inhibition according to the zone gave an idea regarding qualitative efficacy of the test compounds.

Results

The *in vitro* antibacterial study was carried out against the test organisms *Escherichia coli*, *Staphyloccocus aureus*. Aqueous, methanolic and ethanolic extracts of leaves of *Andrographis paniculata* were used for the purpose. The detailed result is depicted in table. 1.

It was observed that all the aqueous extracts were ineffective against the test bacterial organisms. Antibacterial discs prepared from aqueous extracts of leaves of *Andrographis paniculata* at a concentration of 500mg/ ml, 750mg/ml and 1000mg/ml failed to show any zone of inhibition even after 48 hours of incubation.

The ethanolic extract of *Andrographis paniculata* leaves showed varied result at alternate concentrations. While discs charged with 500mg/ml, 750mg/ml plant extracts failed to demonstrate any zone of inhibition against the test organisms, discs charged with 1000mg/ml of plant extracts showed subdue results. The said plant extract at the said concentration showed a zone of inhibition (Mean ± SD) of 6.16 ± 0.114 mm

& 6.98 ± 0.130 mm against tested *Escherichia coli*, *Staphyloccocus aureus* strains respectively after quintuplicate tests.

The methanolic extract of *Andrographis paniculata* leaves at a test concentration of 500mg/ml showed no zone of inhibition, while at a concentration of 750mg/ml and 1000mg/ml zone of inhibition was quite evident. Plant leaf methanolic extract charged @ 750mg/ml in the disc showed a zone of inhibition amounting 6.16 ± 0.089 and 7.08 ± 0.083 mm against *Escherichia coli*, *Staphyloccocus aureus* strains respectively after quintuplicate tests. The methanolic extract of *Andrographis paniculata* leaves at a test concentration of 1000mg/ml showed substantial zone of inhibition of 7.1 ± 0.070 & 7.82 ± 0.130 mm against *Escherichia coli*, *Staphyloccocus aureus* strains respectively after quintuplicate tests.

The zone of inhibition (Mean ± SD) for the test control antibiotic gentamicin (10 mcg/disc) came out as 9.94 ± 0.089 & 9.96 ± 0.089 mm against against *Escherichia coli*, *Staphyloccocus aureus* strains respectively after quintuplicate tests.

The percentage (%) of inhibition of the test organisms were calculated by the given formula for the test compounds (plant extracts).

The percentage (%) of inhibition for *Andrographis paniculata* leaves extracts (ethanolic @ 1000mg/ml) was calculated to be 61.97% and 70.08% against *Escherichia coli*, *Staphyloccocus aureus* respectively. The similar result for methanolic extract (750mg/ml) gave 61.97% and 71.08% of inhibition against *Escherichia coli*, *Staphyloccocus aureus* respectively. The results for higher concentration methanolic extract (1000mg/ml) came out to be 71.42%, 78.51% inhibition against *Escherichia coli*, *Staphyloccocus aureus* respectively. From the above data it was clear that in case of *Andrographis paniculata* leaf, methanolic extract at a concentration of 1000mg/ml found to be more effective against both the test organisms, while ethanolic extract (1000mg/ml) is comparably effective against Staphyloccocus *aureus.*

Table 1: Antibacterial activity of *Andrographis paniculata* leaves extracts

Type of extract	Concentration	Zone of inhibition (mm) (Mean ±SD of quintuplicate)			
		Escherichia coli	**Percentage of inhibition (%)**	***Staphyloccocus aureus***	**Percentage of inhibition (%)**
Ethanolic	500mg/ml	0	-	0	-
	750mg/ml	0	-	0	-
	1000mg/ml	6.16 ± 0.114	61.97	6.98 ± 0.130	70.08
Methanolic	500mg/ml	0	-	0	-
	750mg/ml	6.16 ± 0.089	61.97	7.08 ± 0.083	71.08
	1000mg/ml	7.1 ± 0.070	71.42	7.82 ± 0.130	78.51
Aqueous	500mg/ml	0	-	0	-
	750mg/ml	0	-	0	-
	1000mg/ml	0	-	0	-
Gentamicin	10 mcg/disc	9.94 ± 0.089	-	9.96 ± 0.089	-

Discussion

Aqueous, methanolic and ethanolic extracts of leaves of *Andrographis paniculata* showed nil to effective antibacterial activities at different concentrations. Hossain *et al*. (2021) [8] reported *Andrographis paniculata* efficacy against *Staphylococcus aureus, Escherichia coli* which is similar to the present study findings. Similarly research exercise of Sule *et al*. (2011) [27] reported plant extract (*A. paniculata*) effective against *S. aureus* with a positive zone of inhibition. Researchers like Zaidan *et al*. (2005) [31], Radhika *et al*. (2008) [21], Roy *et al*. (2010) [23], Kataky & Handique (2010) [12] used different types of extracts of *A. paniculata* and reported its activity against methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *E. faecalis* (VRE).

The aqueous extract of the plant failed to show any antibacterial effect. Zaidan *et al*. (2005) [31] also reported the crude water extract of *A. paniculata* leaves exhibited no effect on *E. coli*. Leelarasamee *et al*. (1990) [14] also reported crude powder suspended in water to be devoid of in vitro antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The authors reported *A. paniculata* crude powder suspended in water to be devoid of in vitro antibacterial activity against *Escherichia coli, Staphylococcus aureus* at a concentration of 25 mg/mL crude powder. This finding is similar to the present study report where aqueous extracts could not exhibit antibacterial effects even at a concentration of 1000mg/ml. However, Singh *et al*. (2003) [26] reported the aqueous extract of A. paniculata showed significant antibacterial activity.

The present study advocated for the maximum effectiveness of methanolic extract of leaves which was also reported by Sahalan *et al*. (2007) [24], who reported methanol extract of leaves of *A. paniculata* showed significant activity against *E. coli.*

The ethanolic extract of the plant leaves showed comparable antibacterial activity at higher concentration. Gupta *et al*. (2008) [5] reported 50% ethyl alcohol-treated extracts of *A. paniculata* exhibited antibacterial against *E. coli* which is alike to the present findings. Gupta *et al*. (2008) [5] also reported the potent inhibitory effect of ethanol extract of aerial parts of *A. paniculata* on the growth of *S. aureus, E. coli* along with several other gram positive and negative bacteria.

Andrographis paniculata leaf extracts (aqueous, methanolic, ethanolic) were prepared and used for antibacterial potential determination by disc diffusion method which demonstrated that the plant methanolic and ethanolic leaves extracts have antibacterial potential while aqueous extract lack any such activity.

Conclusions

The present study evinced that the plant *Andrographis paniculata* has antibacterial effects against *Escherichia coli, Staphylococcus aureus* at certain concentrations. The aerial part of the plant, especially leaves can be utilized for the antibacterial use and replacing chemical therapeutic agents in long run. Even so, more studies are required to ascertain the safest concentration and extracts which can be used for

topical and systemic administrations.

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Conflict of Interest
The authors declare no competing or conflict of interest.

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