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ANTIBACTERIAL ACTIVITY OF *ANDROGRAPHIS PANICULATA* OF METHANOLIC EXTRACT AGAINST SOME HUMAN PATHOGENIC BACTERIA AND EFFECT OF ANDROGRAPHOLIDE COMPOUND AGAINST BACTERIAL PATHOGEN

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ABSTRACT: Cold and hot methanolic extract of leaves and whole plant parts of *Andrographis paniculata* were tested against gram-negative *Escherichia coli* (MTCC 1679) *Klebsiella pneumonia* (MTCC 4032) *Streptococcus pyogenes, Solmonella typhimurium* (MTCC733) and gram-positive *Staphylococcus aureus* (MTCC737). Maximum activity among all the bacterial cultures tested was showed against *E. coli* MTCC1679 when compared with their standards. Next to *E. coli*, MTCC1679 best inhibition was seen against *S. aureus* (MTCC737). There was no inhibition reported against, *K. pneumonia, S. pyogenes, Salmonella typhi.* The studied result revealed the highest activity in 75% methanolic extract. The highest zone of inhibition (22 mm) was observed against *E. coli* at a dose of 3.0 mg. The antibacterial activities of 75% methanol extract from *A. paniculata* leaves were observed only against the *E. coli*. The extract was not found active against other tested bacterial pathogens. The Andrographolide compound was analyzed by HPLC chromatogram and the standard of the given andrographolide compound was also prepared by the same.

INTRODUCTION: *Andrographis paniculata* (Bumt.) member of family Acanthaceae also named as "king of Bitters (English) or Kalmegh (Hindi). Plant parts were used by tribes from the time immemorial for a variety of ailments. Commonly used against common cold [1]. *A. paniculata* believed to be used for most biological activities concerned with this plant [2]. Andrographolide as having widely pharmacological properties mostly beneficial as anti-inflammatory [4, 5, 6], antidiabetes [7, 8, 9], antiviral [10, 11, 12], antimalarial [13, 14].

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Hepatoprotective [15, 16, 17], anticancer [18] antihuman immunodeficiency virus (HIV) [19], Immuno-stimulatory [20, 22, 23] and anti snakebite activity [24]. It is effective both internally and externally for skin [25, 26]. Andrographolide inhibits oxidized LDL-induced cholesterol accumulation and foam cell formation in macrophages [27].

Biocompounds having great importance in plants are alkaloids, tannin, flavonoid and phenolic compounds [28]. The tendency of infection has increased to a greater extent and has achieved resistance to a greater extent, become an ever-increasing therapeutic problem [29]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [30, 31]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects

that are often associated with synthetic anti-microbials [32].

Andrographis as an important herb used against the heat of body fever and to destroy toxins from the body [33]. The pharmacology of India reported that it is constituent of twenty-six ayurvedic formulations [34, 35]. *A. paniculata* root and stem extracts were tested for their antimicrobial activity against some bacteria like *Staphylococcus aureus* (NCIM 5021), *Bacillus subtilis* (NCIM 2439), gram-negative bacteria *Escherichia coli* (NCIM 2067), *Proteus vulgaris* (NCIM 2027) and antifungal activity against *Aspergillus niger* (NCIM 1055), *Penicillium chrysogenum* (NCIM 722) by cup plate method [36].

MATERIALS AND METHODS:

Collection and Preparation of the Plant Material: The plant investigated in the present work is widely found in Madhya Pradesh, India. The plant samples were collected from the Kundam block of Jabalpur district in the month of October 2018. The plant identified with the help of flora of Jabalpur and further authenticated at State forest research institute Jabalpur Department of Botany, under voucher no. 16494.

Extraction of Plant Material: The extraction of plant material was done according to the standard method reported by [37]. The selected shade-dried (for 3 to 10 days) plant part leaves were grinded to a fine powder. The powdered plant material was put into Soxhlet for successive extraction with different solvents like ethyl acetate, methanol, and aqueous water. For extraction 25 gm each of the powdered parts was taken with 250 ml of different solvents. The obtained liquid extracts were subjected to a rotary evaporator and subsequently concentrated under reduced pressure (in vacuum at 4 °C) and evaporated to dryness and stored at 4 °C in airtight bottle.

Hot Methanol Extraction: The shade dried coarse powder of the leaves and whole plant material of *Andrographis paniculata* (50 gm each) was packed well in the Soxhlet apparatus and was subjected to methanol by continuous hot extraction for about 24 h. The extracts were filtered through Whatman filter paper and concentrated on a water bath. The final concentrated extracts were stored at -18 °C in labeled sterile bottles.

Cold Methanol Extraction: The shade dried coarse powder of the leaves and whole plant material of *Andrographis paniculata* (50 gm each) was kept in the stoppered flask and were macerated with 250 mL of methanol for 24-48 h with frequent stirring. Then the extracts were filtered through Whatman filter paper and concentrated under air. Obtained extracts were stored at -18 °C in labeled sterile bottles.

Test Microorganisms: Five bacterial cultures namely *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Staphyloccocus aureus* were used in this investigation. All the cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The media used for the antibacterial test were Nutrient Agar/Broth and Muller Hinton agar. All the media were obtained from (HiMedia Pvt Ltd), Mumbai, India.

The test bacterial strains were inoculated into nutrient broth and incubated at 37 °C for 24 h. After the incubation period, the culture tubes were compared with the turbidity (opacity) standard.

Antibacterial Assay: Bioassay was carried out by Agar well diffusion method [10, 11, 12]. Fresh bacterial culture of 0.1 ml having 10^8 CFU was spread on a nutrient agar plate with glass spreader.

A well of 6 mm diameter was punched off into agar medium with a sterile cork borer and filled with 50 µl of aqueous and methanol extracts by using a micro pipette in each well in aseptic condition. Plates were then kept in a refrigerator to allow pre-diffusion of extract for 30 min and further incubated in an incubator at 37 °C for 24 h. The antibacterial activity was evaluated by measuring the zone of inhibition.

The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated. Antibiotic Tetracycline at a concentration of 30 µg/ml as positive control and 100% DMSO (Dimethyl sulphoxide) as negative control were used.

Microorganisms: Cold and hot methanolic extract of leaves and whole plant parts of *Andrographis paniculata* were tested against gram-negative (*Escherichia coli* "MTCC-452") and gram-positive

(*Staphylococcus aureus* "MTCC-737") bacteria. The test organisms were subcultured at 37 ± °C for 24 h and maintained on nutrients agar media. The experimental conditions (temperature and aeration) were maintained constant before the anti-microbial assay was carried out.

Phytochemical Screening through HPLC for Screening of Antibacterial Compound of Andrographolide used against the Tested Bacteria: 1 gm dried powder along with 50 ml of methanol was kept in Soxhlet for one hour. After one hour the refluxing load was subjected to Rota-vapor at 60 RPM and heated at 60 °C. Filter & subject the marc for another two cycles of refluxes (1 h each) with methanol (50 ml) combine with the filtrate. Evaporate under vacuum to dryness Dissolve the residue 10 mg in methanol (10 ml). Filter, Inject the solution in HPLC with the help of 20 µl fixed loop injector and percent content of andrographolide were estimated by counting the area of andrographolide peak in HPLC chromatogram in all sample. HPLC instrument that was used for the estimation of andrographolide were of the following features, HPLC- grade waters, Pump - 515 Isocratic pump, Injector - Rheodyne injector with a 20-microlitre loop, Detector - UV Vis detector, Software - Data ace software, Column - Thermo C-18 column (4.6 × 250 mm, 5 µ particle size), sample size (20 µl). Isocratic elution was carried out with methanol at a flow rate (1 ml/min). The detection was performed with wavelength (230 nm) and column temperature was ambient (30 °C). Class VP software was used for integration and calibration. Evaluation was *via* peak areas with linear regression.

RESULTS:

TABLE 1: ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACTS OF *ANDROGRAPHIS PANICULATA* AGAINST SOME HUMAN PATHOGENIC BACTERIA

Name of the pathogen	Zone of inhibition in mm			
	Hot methanolic extract		Cold methanolic extract	
	M1	M2	M1	M2
E. coli	20 ± 0.5	16 ± 0.5	15 ± 0.5	12 ± 1.0
S. aureus	17 ± 0.5	14 ± 0.5	13 ± 0.5	10 ± 0.5
K. pneumonia	-	-	-	-
P. aeruginosa	-	-	-	-
S. pyogenes	-	-	-	-
S. typhi	-	-	-	-
P. vulgaris	-	-	-	-

M1 = only leaves, M2 = Whole plant ± (SD) - (Absent)

Andrographis paniculata extracted in hot methanolic solvent showed significant activity against all tested bacterial organism than that of cold methanolic extract **Table 1.** Maximum activity among all the bacterial cultures tested was showed against *E. coli* MTCC1679 when compared with their standards. Next to *E. coli*, MTCC1679 best inhibition was seen against *S. aureus* MTCC737. There was no inhibition reported against *K. pneumonia* MTCC4032 *S. pyogenes, S. typhimurium* MTCC733 and *P. vulgaris* at all. Primary screening test revealed that the methanolic extract of *A. paniculata* leaves posses best inhibition against *E. coli* MTCC1679. Leaves of *A. paniculata* were extracted used at different concentrations of methanol solvent and were tested for antibacterial activity. The studied result revealed the highest activity in 75% methanolic extract **Table 2**. The highest zone of inhibition (22 mm) was observed against *E. coli* MTCC1679 at the dose of 3.0 mg. The antibacterial activities of 75% methanol extract from *A. paniculata* leaves were observed only against the *E. coli* MTCC1679. The extract was not found active against other tested bacterial pathogens.

TABLE 2: ANTIBACTERIAL ACTIVITY OF *A. PANICULATA* LEAF EXTRACT PREPARED WITH DIFFERENT PERCENTAGE OF METHANOL

Different extract of *A. paniculata* leaves	Amount of extract	Zone of inhibition in mm against *E. coli* MTCC1679
25% methnaolic extract	100µl=1.0 mg	ND
50% methanolic extract		10
75% methanolic extract		22
100% methanolic extract		12

ND = Not detected

FIG. 1: ANTIBACTERIAL ACTIVITY OF ANDROGRAPHOLIDE COMPOUND EXTRACTED FROM TLC PLATE HAVING R_f VALUE 0.39 AGAINST BACTERIAL ISOLATE *E. COLI* MTCC1679

Antibacterial activity of the isolated compound having a zone of inhibition in the range of 12 mm against the bacterial pathogen *E. coli* MTCC1679 determines the antibacterial activity in the normal range **Table 3**.

This andrographolide compound was analyzed by the HPLC chromatogram and the standard of the given andrographolide compound was also prepared by the same.

TABLE 3: ANTIBACTERIAL ACTIVITY OF ANDROGRAPHOLIDE COMPOUND AGAINST BACTERIAL PATHOGEN *E. COLI* MTCC1679

Bacterial strain	Amoxicillin Positive control	Activity of isolated metabolites
E. coli MTCC1679	24 ± 0.5	12 ± 1.0

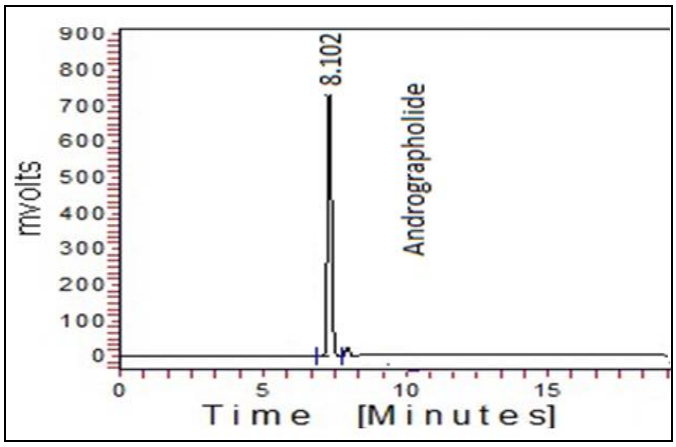

FIG. 2: HPLC CHROMATOGRAM OF STANDARD ANDROGRAPHOLIDE ANALYZED FROM THE METHANOLIC EXTRACT OF *A. PANICULATA*

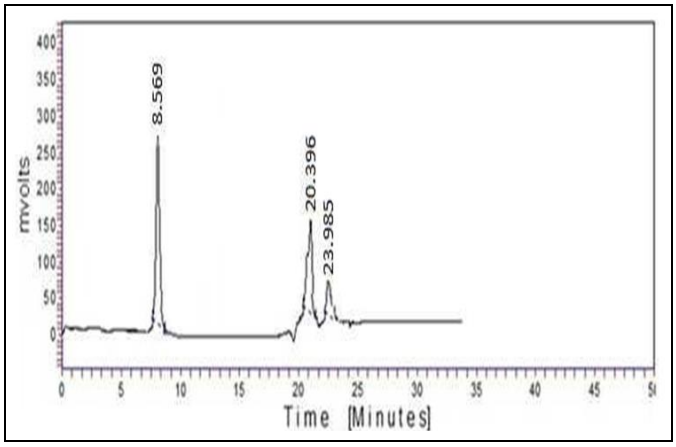

FIG. 3: PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF *A. PANICULATA* FOR ISOLATION OF ANTIBACTERIAL COMPOUND ANDROGRAPHOLIDE BY HPLC METHOD

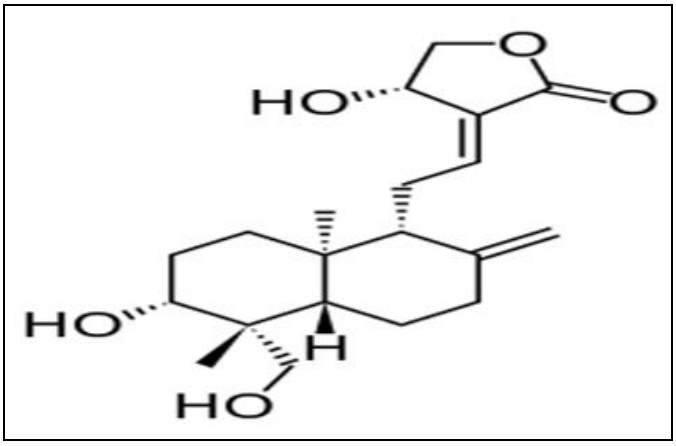

FIG. 4: ANDROGRAPHOLIDE COMPOUND

DISCUSSION: Microbiology in the present scenario faces many critics concerning resistance, superbugs and the prospects of a post-antibiotic era. The threat of drug resistance forced the continuous development of new drugs or antibiotics. But the decline in the field of industrial and academic research results in current rate of discovery of new antibiotics lower than in the golden age of antibiotics in the (the 1940s-1960s) [38]. In the present study, *A. paniculata* methanolic extract exhibited inhibitory activity against the growth of *E. coli* and *S. aureus* and there was no inhibitory activity found against other studied microorganism. This result was similar and supported by many researchers who already reported that *A. paniculata* as potent antimicrobial activator [39] reported that 75% methanol extract of *A. paniculata* leaves was found to be active against *S. aureus* and *E. coli* [40]. studied the aqueous extract of *A. paniculata* antibacterial activity towards both gram-negative and gram-positive bacteria. The leaves of *Andrographis paniculata* showed the best antibacterial activity against *S. aureus* and *E. coli*. A low degree of inhibition was observed against *B. licheniformis, K. pneumonia, S. pyogenes, S. typhi, P. vulgaris* and *A. hydrophila* at all.

CONCLUSION: Microbiology in the present scenario faces many critics concerning resistance, superbugs and the prospects of a post-antibiotic era. The threat of drug resistance forced the continuous development of new drugs or antibiotics. But the decline in the field of industrial and academic research which results current rate of discovery of new antibiotics lower than in the golden age of antibiotics methanolic crude extract of *Andrographis paniculata* best shows anti-microbial activity for the more resistant type of microorganism.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

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