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ANTIBACTERIAL ACTIVITY OF METHANOL LEAVES EXTRACT OF *ANDROGRAPHIS PANICULATA* AND PASS PREDICTION FOR ANTIBACTERIAL EFFECT AND ADME/T PROPERTY ANALYSIS OF ITS ISOLATED COMPOUNDS.

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ABSTRACT

The present study aims to investigate the antibacterial effect of methanol extract of *Andrographis paniculata* leaves by disk diffusion method and *in silico* PASS prediction used for six phytoconstituents namely 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide isolated from *A. paniculata*. And also ADME/T properties of the phytoconstituents were analyzed using Qikprop 3.2 module. The extract indicated zone of inhibition against Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) and Gram negative bacteria (*Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli*). Gram negative bacteria *Pseudomonas aeruginosa* demonstrated no action against *A. paniculata* leaves extract. Relative percentage inhibition of the extract against each bacterium also calculated. In the PASS prediction for their thrombolytic activity of the isolated phytoconstituents, we found wide range of activity. Among all the phytoconstituents, 14-acetylandrographolide showed highest Pa for antibacterial activity (Pa=0.631). After 14-acetylandrographolide, isoandrographolide may be the second choice. So, 14-acetylandrographolide and isoandrographolide may be

competitive candidate for promising antibacterial agent. From the ADME profiles of 14-acetylandrographolide and isoandrographolide, it cleared that they are safe for human. Further *in vivo* investigation need to identify whether 14-acetylandrographolide, isoandrographolide and other compounds have antibacterial effect or not.

KEYWORDS: *Andrographis paniculata*, Antibacterial, PASS prediction, ADME/T properties, 14-acetylandrographolide, isoandrographolide.

1 INTRODUCTION

The discovery of anti-microbials like penicillin from *Penicillium notatum*, various other antibiotics have been initiated the search for naturally available bioactive molecules from living organisms.[1] Many of the bioactive molecules are secondary metabolites, generated in response to external pressures such as competition for space and potential predators. According to the World Health Organisation[2], 65% of the world's population has incorporated ethnomedicine in their primary health care practice. In some African and Asian countries, 80% of the population depends on traditional medicine for primary health care and about 70% of population in the developed world has used alternative or complementary medicines. For example, of the plants used to treat microbial infections, an estimated 6% have been screened for specific anti-microbial activities and only a small proportion of these have been studied Phytochemical to identify the active constituents and/or blends.[3, 4] So, if a plant found with great antibacterial activity and no resistant to bacteria, then it could be great finding.

Prediction of activity spectra for substances (PASS) is hosted by the V. N. Orechovich Institute of Biomedical Chemistry under the aegis of the Russian Foundation of Basic Research. The webbased application predicts the biological activity spectrum of a compound based on its structure. It works on the principle that the biological activity of a compound equates to its structure. PASS prediction tools are constructed using 20000 principal compounds from MDDR database (produced by Accelrys and Prous Science). The database contains over 180000 biologically relevant compounds and is constantly updated.[5, 6]

ADME is an abbreviation in pharmacokinetics and pharmacology for "absorption, distribution, metabolism, and excretion," and describes the disposition of a pharmaceutical compound within an organism. The four criteria all influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological

activity of the compound as a drug. Many server and software are now available for prediction of this ADMET properties of compounds.[7]

Andrographis paniculata (Burm. f.) Nees (Acanthaceae) (*A. paniculata*, *Chuanxinlian*), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection.[8, 9] The aerial part of *A. paniculata* is commonly used in Chinese medicine. According to Chinese medicine theory, *A. paniculata* 'cools' and relieves internal heat, inflammation and pain and is used for detoxication.[10, 11] Many phyto constituents isolated from *A. paniculata*[12] and six of them are 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide.

The aim of the present study to evaluate the antibacterial effect of methanol extract of *Andrographis paniculata* leaves and to predict whether the isolated compounds from *A. paniculata* had antibacterial effect, which was done by using *in silico* tools PASS prediction. And also ADME/T properties of the phytoconstituents were analyzed using Qikprop 3.2 module.

2 MATERIALS AND METHODS

2.1. Collection and identification of plant material

The leaves of *Andrographis paniculata* were collected from Chittagong area in November, 2014 then identified by Dr. Sheikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh.

2.2. Preparation of Extract

The collected leaves were washed thoroughly with distill water, chopped, air dried for a week and pulverized in electric grinder. The powder (700 g) obtained was successively extracted in methanol (55-60ºC) for 10 days with a 2 days interval. The filtrated supernatant was evaporated to dry using a rotary evaporator (RE200, BB Sterling, UK) under reduced pressure. The crude extract (blackish green semisolid, yield 8%) was preserved at 4ºC until further use.

2.3 Chemicals

All chemicals used were of analytical reagent grade. Methanol was purchased from Merck, Germany. Kanamycin (30µg/disc, Oxoid, England) was used as a standard antibiotic disc.

2.4 *In vitro* Antibacterial activity

2.4.1 Bacterial strains

Seven bacterial species, gram-positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* gram-negative *Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa*. These microbes were obtained from the department of Pharmacy International Islamic University Chittagong.

2.4.2 Media preparation and maintenance of bacteria

All of the bacterial strains were grown and maintained on Nutrient agar (Merck, India) media at 37 °C and pH (7.4±0.2). The bacteria were subculture overnight.

2.4.3 Preparation of concentration

In the study of the antibacterial activity, the extract was diluted in methanol. The concentrations to the extract given in Table 1 are expressed in terms of µg/ disk.

2.4.4 Preparation of discs

The sample discs of about 5 mm in diameter were cut by punching machine (Kangaro 280) from Whatman No. 1 filter paper (Made in China). The discs were taken in a Petri dish and sterilized by autoclave (Daihan Labtech Co., LTD Model: LIB-060M: ISO 9001 certified) dried in oven at 180°C.

2.4.5 Antibacterial screening by disk diffusion technique

The antibacterial assay was performed by using the disc diffusion method [Barry.,1980; Bauer.,1996]. Seven pathogenic bacteria were used as test organisms for antibacterial activity of *A. paniculata* leaves methanol extract. The test organisms were inoculated on 10 ml previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile Petri dish in an aseptic condition using a sterile loop. Prepared sample and standard solutions were applied to the corresponding Petri dish. The plates were incubated for overnight at 37^0 C. After proper incubation, clear zone of inhibition around the point of application of sample solution were measured which is expressed in millimeter (mm).

2.4.6 Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula.[13]

Relative percentage inhibition of the test extract

$$\frac{100 \times (x - y)}{(z - y)}$$

Where,

x = total area of inhibition of the test extract

y = total area of inhibition of the solvent

z = total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition.

2.5 Statistical Analysis

The results were expressed as mean±SD from triplicate experiment for zone of inhibition from triplicate experiments for Antibacterial activity. The results obtained were compared with the negative control group for antibacterial activity and $P < 0.05$, $P < 0.01$ and $P < 0.001$ was considered to be statistically significant in Dennett's tests.

2.6 *In silico* Prediction of activity spectra for substances (PASS)

Prediction of phytoconstituents namely 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide isolated from *A. paniculata* [12] for antibacterial activity was done with the help of computer program, PASS (Prediction of activity spectra for substances). Software estimates predicted activity spectrum of a compound as probable activity (P_a) and probable inactivity (P_i). The prediction of activity is based on structure-activity relationship analysis of the training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities. The values of P_a and P_i vary between 0.000 and 1.000. Only activities with $P_a > P_i$ are considered as possible for a particular compound. If $P_a > 0.7$, the probability of experimental pharmacological action is high and if $0.5 < P_a < 0.7$, probability of experimental pharmacological action is less. If the value of $P_a < 0.5$, the chance of finding the activity experimentally is less, but it may indicate a chance of finding a new compound.[5, 14-16]

2.7 ADME/T property analysis

Ligand based ADME/Toxicity prediction

The QikProp module of Schrodinger (Maestro, version 10.1) is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program design to produce certain descriptors related to ADME. It predicts both physicochemical significant descriptors and pharmacokinetically relevant properties. ADME properties determine drug-like activity of ligand molecules based on Lipinski's rule of five. ADME/T properties of the compound (DIM) was analyzed using Qikprop 3.2 module.[17]

3 RESULTS

3.1 *In vitro* Antibacterial activity

Antibacterial activity of the extract was tested against seven pathogenic bacteria and were compared with the standard antibiotic Kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm) showed in Table 1 and Relative percentage inhibition of the test extract presented in Table 2.

Table 1: Antibacterial activity of Methanol extract of *A. paniculata* leaves

Name of the bacteria	Diameter of zone of inhibition (mm)		
	Methanol extract of *A. paniculata* leaves		Standard (Kanamycin) (30µg/disc)
	700µg/disc	1000µg/disc	
Gram Positive			
Staphylococcus aureus	11.0±1.0[b]	13.0±0.5[b]	22.2±0.76
Bacillus subtilis	9.2±1.04[a]	12.0±1.0[b]	18.2±0.29
Bacillus cereus	10.5±1.04[a]	14.0±1.0[b]	25±0.50
Gram Negative			
Salmonella typhi	7.0±1.04[a]	9.0±1.0[b]	25.3±0.58
Salmonella paratyphi	8.5±1.04[a]	11.0±1.0[b]	20.3±0.29
Escherichia coli	13±1.0[a]	16.0±1.0[b]	23.5±0.50
Pseudomonas aeruginosa	-	-	25.5±0.50

Values are mean inhibition zone (mm) ± S.D of three replicates. The different superscripted (a, b) values have significantly different ([a]P < 0.01 and [b]P < 0.001) as compared with standard (Kanamycin) in same row in Dunnett's test by SPSS. - - - = no zone of inhibition.

Table 2: Relative percentage inhibition of Methanol extract of *A. paniculata* leaves with their doses compare to standard antibiotics.

	Relative percentage inhibition (%)	
Name of the bacteria	**Methanol extract of *A. paniculata* leaves**	
	700µg/disc	**1000µg/disc**
Gram Positive		
Staphylococcus aureus	24.6	34.4
Bacillus subtilis	25.6	43.6
Bacillus cereus	17.6	31.4
Gram Negative		
Salmonella typhi	7.64	12.6
Salmonella paratyphi	17.5	29.3
Escherichia coli	30.6	46.4
Pseudomomas aeruginosa	-	-

Values calculated from their mean values.

3.2 *In silico* PASS prediction

Six phytoconstituents namely 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide were analyzed by the PASS for their antibacterial activity and results were used in a flexible manner. All the compounds showed greater Pa than Pi (Table 3). 14-acetylandrographolide showed highest Pa for antibacterial activity (Pa=0.631).

Table 3: PASS predictions of 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide for antibacterial activity.

Phyto compounds	**PASS predictions for antibacterial activity**	
	Pa	**Pi**
5-hydroxy-7,8-dimethoxyflavone	0.387	0.033
14-acetylandrographolide	0.631	0.007
14-deoxyandrographolide	0.451	0.022
andrograpanin	0.315	0.054
isoandrographolide	0.551	0.012
neoandrographolide	0.531	0.014

3.3 ADME and Toxicity analysis

Ligand based ADME/Toxicity prediction

The drug-like activity of the ligand molecule was categorized using ADME properties by QikProp module of Schrodinger. The ADME property of the 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide were evaluated with QikProp module of Schrodinger, shown in Table 4. The selected properties are known to influence metabolism, cell permeation, and bioavailability. All the predicted properties of the isolated compound was in the range for satisfy the Lipinski's rule of five to be considered as drug like potential.

Table 4: ADME/T properties of 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide by QikProp.

Name of Molecules	MW[α]	HB donor[β]	HB acceptor[€]	LogP[¥]	Molar Refractivity[µ]
5-hydroxy-7,8-dimethoxyflavone	297.0	0	5	2.264770	69.54
14-acetylandrographolide	408.0	2	6	3.026692	127.81
14-deoxyandrographolide	348.0	3	4	3.422611	115.67
andrograpanin	332.0	1	3	2.673059	91.11
isoandrographolide	366.0	3	5	4.513255	92.52
neoandrographolide	506.0	5	8	2.940555	124.83

[α]Molecular weight (acceptable range: <500).

[β]Hydrogen bond donor (acceptable range: ≤5).

[€]Hydrogen bond acceptor (acceptable range: ≤10).

[¥]High lipophilicity (expressed as LogP, acceptable range: <5).

[µ]Molar refractivity should be between 40-130.

4 DISCUSSIONS

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Plant has long been a very important source of drug and many plants have been screened whether they contain compounds with therapeutic activity.[18] Therefore, it is vital to evaluate the antimicrobial activity of *A. paniculata*. The first step towards this goal is the *in vitro* antibacterial activity assay.[19] The bacterial strains were chosen to be studied as they are important pathogens and rapidly develop antibiotic resistance as antibiotic use increases. In disc diffusion technique, the mean zone of inhibition produced by the commercial antibiotic, tetracycline, was larger than those produced by ethanol extract.

It may be attributed to the fact that the plant extract being in crude form contains a smaller concentration of bioactive compounds.[20] In classifying the antimicrobial activity it would be generally expected that much greater number would be active against Gram positive than Gram negative bacteria.[21] However, in this study, the plant extract was effective against both Gram positive and Gram negative bacteria suggesting the presence of broad spectrum of antibiotic compounds or simply general metabolic toxin in the plant extract.[22]

In order to accelerate the research for potent natural products, computer-aided drug discovery program PASS was used to predict the biological activity. PASS prediction tools were constructed using 20000 principal compounds[23] and about 4000 kinds of biological activity on the basis of structural formula with mean accuracy about 90%.[24] The result of prediction is presented as the list of activities with appropriate Pa and Pi ratio. Six phytoconstituents namely 5-hydroxy-7, 8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide were analyzed by the PASS prediction for their antibacterial activity and found wide range of activity. 14-acetylandrographolide was the best compound for antibacterial effect from all the compounds, though it had bigger Pa value (0.631). According to Pa value, the compounds showed antibactrial effect as following,
14-acetylandrographolide > isoandrographolide > neoandrographolide > 14-deoxyandrographolide > 5-hydroxy-7,8-dimethoxyflavone > andrograpanin.

In our study, we also tried to explore out the ADME and toxicity profiles all of six compounds, i.e., 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide. As described in Table 4, the ADME profile of all the compounds had wide range, but all were within the acceptable range. From the ADME profiles of all examined compounds, it cleared that they are safe for human.

5 CONCLUSION

From the study it was found that, *A. paniculata* could be great source of new antibacterial drugs. PASS prediction showed highest value of Pa (Probability of activity) for 14-acetylandrographolide. The data support that 14-acetylandrographolide f is the best compounds against bacteria at wide range, as it possessed higher value in PASS prediction. After 14-acetylandrographolide, isoandrographolide may be the second choice. So, 14-acetylandrographolide and isoandrographolide may be competitive candidate for promising

antibacterial agent. From the ADME profiles of 14-acetylandrographolide and isoandrographolide, it cleared that they are safe for human. Further *in vivo* investigation need to identify whether 14-acetylandrographolide, isoandrographolide and other compounds have antibacterial effect or not.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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