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Investigating the Potential of Andrographis Paniculata as a Natural Protection Against Pathogens for Gentle and Intimate Care

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KEYWORDS

Urinary tract infection, *Andrographis paniculata*, antibacterial, gram-negative, gram-positive, MTT assay.

ABSTRACT:

Introduction: Bacterial infections present a substantial worldwide challenge to public health, causing chronic diseases and fatalities. The proliferation of antibiotic-resistant strains, driven by misuse of antibiotics, has intensified this issue. Consequently, multidrug-resistant (MDR) pathogens have emerged, causing diverse bacterial infections and resulting in increased morbidity and mortality rates. As a precautionary measure, this study aimed to explore the potential of Andrographis paniculata, a medicinal plant, and its derived product against infection causing pathogens.

Objectives: The primary aim of this study was to investigate the antibacterial properties and formulation of a herbal soap utilizing extracts from the leaves of Andrographis paniculata, targeting a range of pathogenic bacteria. Additionally, the study sought to assess the biocompatibility of the herbal soap through MTT assay analysis.

Methods: The leaf extracts of Andrographis paniculata were obtained using chloroform, methanol, and distilled water as solvents. Phytochemical analysis and antioxidant activity was performed. The antibacterial activity of these extracts was tested against both gram-positive and gram-negative pathogens including P. aeruginosa, S. aureus, E. coli and B. licheniformis by Agar well diffusion and Minimum Inhibitory Concentration methods. Furthermore, a formulation of the hygiene product, a herbal soap was developed and its antibacterial activity as well as biocompatibility through MTT assay was evaluated for the analysis of its potential, safety and efficacy.

Results: The phytochemical studies specified the existence of various phytochemicals in different extracts such as alkaloids, flavonoids, tannins, saponins. The extracts showed potent antioxidant activity. Significant antimicrobial activity was shown by the crude extracts and herbal soap product on the bacterial pathogens at different concentrations. The MTT assay results showed that further reduction in drug dosage of the herbal soap product may lead in the improvement of cell viability. The cytotoxicity of the herbal soap was dose dependent.

Conclusions: The promising results of this study pave the way for future advancements in the development of herbal products derived from Andrographis paniculata leaf extracts, particularly in their efficacy against infectious pathogens.

Journal of Chemical Health Risks

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1. Introduction

The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have noticeably endangered the current antibacterial therapy. Bacterial infections resistant to multiple drugs (MDR) frequently result in elevated mortality rates, extended hospital stays, and escalated treatment expenses, exacerbating the burden on healthcare systems [1-2]. The most problematic bacteria include, pathogens like E. coli, Staphylococcus aureus, Klebsiella, Pseudomonas aeruginosa, Bacillus licheniformis etc [3-4]. Bacterial infections that are resistant to multiple drugs (MDR) often lead to higher mortality rates, prolonged hospitalization, and increased healthcare costs, further straining healthcare resources. Many organisms have developed resistance to antibiotics, leading to the emergence of multidrug-resistant pathogens. The indiscriminate use of antibiotics has worsened this problem. The progress of drug confrontation as well as appearance of unwanted side effects of certain medications has led to the exploration of new-fangled antimicrobial agents from medicinal plants particularly [5-6].

Plants have served as a crucial medicinal resource for centuries, appreciated not only as nutritional supplements but also for their traditional use in treating various health ailments. Despite significant progressions in the synthesis of artificial drugs, numerous pharmaceutical drugs in manufacture are directly derivative from plants. The utilization of medicinal plants is on the rise in developing countries, offering a novel source of antibacterial, antifungal, and antioxidant agents. In recent years, there has been a resurgence in the utilization of herbal products, possibly powered by increasing consumer discontent with conventional medicines, advancements in chemical, pharmacological, and clinical assessments, the evolution of newer dosage forms, and the rise in self-medication practices [7-9]. Herbal Medicinal Products (HMP) are those medicinal products that contain exclusively as ingredients herbal drugs (e.g., parts of plants) or pharmaceutical preparations there of (e.g., extracts, essential oils, etc.) The World Health Organization (WHO) also deliberates phytotherapy in its wellbeing agendas recommending basic measures for authentication of medicines and products from herbal source in evolving countries. WHO has also issued monographs regarding the value, safety, as well as efficiency of designated therapeutic plants as well as commendations on their cultivation, along with on the quality control, efficacy and safety of herbal medicinal products [10-11]. Andrographis paniculata (Burm.f.) (Family Acanthaceae) an herbaceous plant mainly cultivated in Southern Asia, China as well as in some parts of Europe. In outmoded medicine, A. paniculata is used to lower the body temperature, eradication of the contaminants from the body, prevention of common cold, treating upper respiratory tract contagions counting sinusitis and fever [12-14]. The typical secondary metabolites found in this plant consists significantly improved its magnitude amid the remedial plants. The intention of the current study was to explore the antibacterial activity and formulation of an herbal product as soap consisting the leaves extracts of Andrographis paniculata contrary to infection causing pathogens. The biocompatibility of the herbal soap was also analysed by means of MTT assay to ensure its safety and efficiency for future aspect.

2. Objectives

The primary aim of this study was to investigate the antibacterial properties and formulation of a herbal soap using extracts from Andrographis paniculata leaves, targeting various pathogenic bacteria. The study also aimed to assess the biocompatibility of the herbal soap through MTT assay analysis. This included identifying the antibacterial efficacy of the extracts, optimizing the soap formulation for maximum effectiveness, and ensuring the safety and compatibility of the product with human cells. The ultimate goal was to develop a safe, effective, and natural antibacterial soap suitable for personal hygiene and pathogen protection.

3. Methods

3.1 Collection of bacterial samples

Bacterial samples including the Escherichia coli culture MTCC 1687, Pseudomonas aeruginosa culture MTCC 12011, Staphylococcus aureus culture MTCC 10787 and Bacillus licheniformis culture MTCC 11813 were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) located in Chandigarh.

3.2 Plant material

The leaves of Andrographis paniculata plants were collected from the region of Chhattisgarh state, India. After gathering the leaves, they were carefully washed

Journal of Chemical Health Risks

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using running tap water to remove any impurities. Subsequently, the leaves were dried under shade to maintain their quality. Once dried, the leaves were finely ground into a powder using an electric blender. The powdered leaves were then stored in airtight containers at room temperature for future utilization.

3.3 Extraction of plant material

To extract the bioactive compounds from the powdered Andrographis paniculata leaves, the powdered samples were subjected to extraction using three different organic solvents methanol, distilled water, and chloroform. The Soxhlet apparatus, operating on a 7-hour cycle, was utilized for this purpose at a temperature of 60 degrees Celsius. The resulting crude extract, obtained after evaporation, was carefully stored in a refrigerator for future use in research or other applications.

3.3 Phytochemical analysis

The crude extract of Andrographis paniculata leaves was subjected to phytochemical analysis to identify the presence of various bioactive constituents. The analysis was conducted using qualitative measures based on established criteria, with some inconsequential modifications [15-20].

3.5 Antioxidant activity by DPPH assay

A stock solution was prepared by dissolving 3.3 mg of DPPH in 100 ml of methanol.3 ml aliquot of this working solution was taken and mixed with the plant extract samples at various concentrations. The reaction mixture and incubated it in the dark for 60 minutes at room temperature. The absorbance of the reaction mixture was measured at 517 nm. The scavenging effect percentage was calculated using the following formula:

[(control absorbance−sample absorbance)/(control absorbance)]×100

3.5 Antimicrobial activity

3.5.1. Agar well Diffusion method

The agar well diffusion method was conducted following the standard procedure. The efficacy of crude leaf extracts was tested against bacterial strains. A 6mm well was created in agar plates, and bacterial strains were swabbed onto the plates using a well cutter. Subsequently, 100µl of plant crude extracts, obtained from different solvents and at various concentrations, were added into the wells. The positive control consisted of an antibiotic, while the negative control involved bacterial culture plates. The plates were then incubated at 37°C for 24 hours.

3.5.2. Minimum Inhibitory Concentrations

In order to determine the minimum inhibitory concentration (MIC) values, the broth dilution method was employed following the prescribed criteria. The test organisms were cultivated in a nutrient broth medium. To begin the experiment, 100µl of plant extracts at varying concentrations was mixed with sterile nutrient broth, and then inoculated with 100µl of bacterial suspension that had been cultured overnight in a broth medium. Following this, the tubes containing the mixture were incubated at 37°C for a duration of 18 hours. Bacterial growth inhibition was assessed by measuring the absorbance at 600 nm using a spectrophotometer. The MIC was determined as the lowest concentration of the extract that effectively hindered bacterial growth [21-22].

3.6 Formulation of Herbal Product

Herbal product was formulated consisting the extracts of Andrographis paniculata and basic ingredients. Its efficacy was analyzed by antimicrobial activity using agar well diffusion and the Minimum Inhibitory Concentration method.

3.7 Physicochemical properties of the formulated soap

A range of physicochemical parameters were assessed to validate the quality of the formulated herbal soap [23-24].

3.7.1. Physical characteristics

The formulated soap was evaluated for its odor, color, and overall appearance.

3.7.1.1. Determination of pH

The pH of a soap solution was determined by dissolving formulated soap in 100 ml of distilled water and measured it using a calibrated pH meter.

3.7.1.2. Moisturizing Ability

The efficacy of the soap in moisturizing was assessed by measuring the skin's hydration level following its application.

3.7.1.3. Foam height

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1943-1958 | ISSN:2251-6727

A sample of soap weighing 0.5g was dispersed in 5 ml of distilled water and transferred to a 10 ml measuring cylinder. After giving it five to ten strokes, the mixture was allowed to stand, and the height of the foam formed above the aqueous volume was measured.

3.7.1.4. Foam retention

1% solution of formulated soap was prepared by dissolving the soap in distilled water. Then, 25 ml of this soap mixture was transferred to a 100 ml measuring cylinder and shaken ten times. The volume of foam was subsequently measured at 1-minute intervals over a period of 10 minutes.

3.7.1.5. Skin Irritation

The test involves applying the soap to the skin for a duration of 10 minutes. If no irritation occurs during this period, the product is classified as non-irritating (patch test).

3.8 Biocompatibility test of product

A biocompatibility test is a test that determines how compatible a material is with living tissue. It's often used to test medical devices, implants, and other materials that will come into contact with the human body.

3.8.1. Cell Viability using MTT assay

5 mg/ml of the herbal soap sample AP was dissolved in autoclaved distilled water and filtered through 0.22µm syringe filter. Sample was serially diluted from 0-2.5mg/ml in 1%FBS media. The MTT based cytotoxic assay for the herbal sample was performed in HaCat, skin epithelial cell line. Five thousand (5x103) cells were seeded in 96 – well plate and were incubated for 48 hrs, before during exposures. 72 hrs post drug treatment, cells were processed for MTT assay. The MTT assay was removed and 100µl of DMSO was added to each well dissolve the formazan crystals 5 times and measured at 570 nm. Cell viability (%) was determined by using the following formula: -

Viability % = [OD (Treated)/OD (control)] * 100

4. Results

In the present study, a preliminary phytochemical analysis was conducted on different extracts of Andrographis paniculata leaves. The presence or absence of various phytochemicals, including alkaloids, flavonoids, saponins, tannins, glycosides, and phenolic compounds, was observed and recorded. Andrographis paniculata holds promise as a natural protective agent against pathogens in gentle and intimate care products. Its antimicrobial, immunomodulatory, and anti-inflammatory properties make it a valuable ingredient for promoting skin health and well-being. Further research and product development efforts are warranted to harness its full potential in this application

4.1 Antioxidant activity by DPPH assay

The results of the DPPH assay revealed that the distilled water and chloroform crude extracts of leaves of A. paniculata showed a higher scavenging potential of 80.4% and 79.5%. as compared to the standard ascorbic acid which was 72%. In contrast, methanol crude extracts did not show a potent inhibition percentage which was 58.4%. Further, the stem crude chloroform and distill water extract showed scavenging potential of 97.55% and 80.408% whereas methanol extract showed a potential of 72.97% as compared to the standard. The chloroform and methanol extract of the stem revealed better scavenging potential in contrast to leaves extract. the study noted that the stem extracts generally showed superior scavenging potential compared to the leaf extracts (Fig. 1). This suggests that the antioxidant compounds present in Andrographis paniculata may vary in concentration or composition between different parts of the plant. The chloroform and methanol extracts from the stems, in particular, demonstrated notable antioxidant activity, indicating their potential as sources of natural antioxidants for various applications, including pharmaceuticals, cosmetics, and functional food.

4.2 Antimicrobial activity by Agar Well Diffusion Method

The antimicrobial susceptibility test revealed significant variations in vulnerability and resistance patterns of bacterial isolates to a specific antibiotic. The test was conducted using the Agar well diffusion method, and the antibacterial effect of plant extracts varied depending on the type of extract and its concentration. The results demonstrated that the pathogens exhibited susceptibility to different crude extracts of Andrographis paniculata leaves. P. aeruginosa displayed the highest sensitivity to the chloroform extract of the leaves at concentrations of 1mg/ml and 10mg/ml whereas, it was found to be sensitive against 1mg/ml, 5mg/ml and 10mg/ml

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1943-1958 | ISSN:2251-6727

concentrations of distill water extracts. With the methanolic extract, it was sensitive at 10mg/ml. Similarly, the chloroform and distilled water extract of leaves exhibited effective antibacterial activity against pathogen S. aureus at 10mg/ml and 5mg/ml. On the other hand, E. coli and B. licheniformis demonstrated greater sensitivity towards the chloroform and distilled water extracts of the leaves at all concentrations (Fig. 2). B. licheniformis was found to be sensitive against methanolic extract with an inhibition zone of 8mm and 12mm at 5mg/ml and 10 mg/ml. It was observed that pathogens were resistant to the methanolic extracts of the leaves and showed greater sensitivity to the chloroform and distilled water extracts (Table 1). Additionally, all pathogens exhibited sensitivity to the antibiotic Norfloxacin, which was used as a positive control. Interestingly, it was observed that some pathogens were resistant to methanolic extracts but showed sensitivity to chloroform and distilled water extracts. This indicates that the choice of solvent for extracting bioactive compounds from plant materials can significantly influence their antimicrobial activity. Moreover, the study included a positive control using the antibiotic Norfloxacin, which demonstrated sensitivity against all tested pathogens. This validates the effectiveness of the Agar Well Diffusion Method and confirms the reliability of the results obtained from the study. Overall, the findings of this study highlight the potential of Andrographis paniculata leaf extracts as alternative antimicrobial agents against a range of bacterial pathogens, and it underscores the importance of further research to elucidate the mechanisms of action and optimize the extraction methods for maximum efficacy.

4.3 Formulation of Herbal Product

An herbal product as soap specifically was formulated and developed and its antibacterial activity was analyzed against the pathogens. The commercial product was taken as the standard for comparative study.

The agar well method was employed to assess the antibacterial activity of the herbal product (soap). The results indicated that P. aeruginosa exhibited sensitivity to the herbal product at a concentration of 5mg/ml. S. aureus showed sensitivity at 1mg/ml and 10mg/ml, further, E. coli displayed sensitivity at a concentration of 10mg/ml. Moreover, the herbal product demonstrated effectiveness at a concentration of 1mg/ml. Whereas, B. licheniformis was found to be sensitive at 1mg/ml and 10mg/ml. Based on these findings, it can be concluded that the herbal product exhibited significant potential in inhibiting the growth of the human pathogens, as compared to the efficacy of standard commercial products.

4.4 Minimum Inhibitory Concentration (MIC)

The broth dilution method was employed to determine the Minimum Inhibitory Concentration (MIC) of the plant extracts and herbal product containing extracts of A. paniculata. The results demonstrated that the different crude extracts and herbal product exhibited MIC activity against both gram-positive and gram-negative pathogens. The MIC values of the methanolic extracts were found to be effective at 3mg/ml respectively against P. aeruginosa. Whereas the chloroform as well as distilled water extract of leaves of A. paniculata showed effective results against S. aureus at the concentration of 1mg/ml and 2mg/ml. MIC values of the chloroform extract of the leaves were detected at 1mg/ml and 5mg/ml against the E. coli. Whereas B. licheniformis was found to be sensitive against chloroform extract at 1mg/ml and 2mg/ml. The result from the present study indicated that the methanolic, chloroform and distilled water extract showed more probable MIC against the pathogens. MIC of herbal product was effective against E. coli at 3mg/ml concentration whereas it was effective at 2mg/ml against P. aeruginosa. The herbal product showed potent MIC value against S. aureus at 3mg/ml concentration (Table 2).

4.5 Physicochemical properties of the formulated soap

The above given table describes the colour, odour, shape, pH, irritation, foam height and foam retention of the herbal soap. The colours of the herbal soap were green. The odour of all was aromatic. As per evaluation the pH of herbily formulated soap is 7.9 which is near about basic and there was no skin irritation found (patch test). Besides that, the foam height was estimated as 3.2 cm. Foam retention of the herbal soap was 5min (Fig. 4). The results of MTT assay revealed that after 72 hours post drug treatment the cytotoxicity was dependent on dose concentration of the herbal soap (AP). The cell viability was lowest at the concentration of 2.5 mg/ml with below 20% value along with visible white precipitations. The percent value of the cell viability was found to increase

Journal of Chemical Health Risks
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with the reduction in concentration of the herbal sample (Fig. 5).

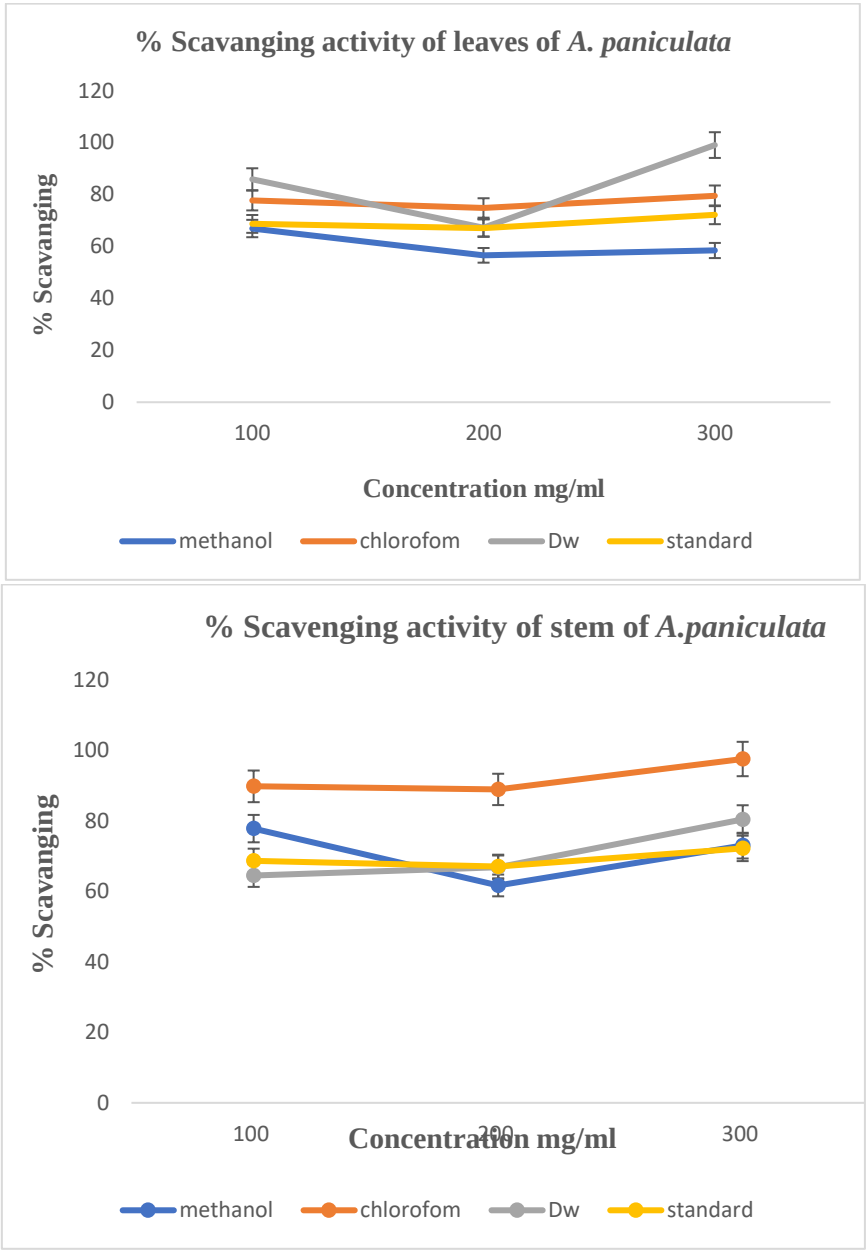

Fig.1 Antioxidant activity of different crude extracts of leaves and stem of A. paniculata by DPPH assay

Journal of Chemical Health Risks

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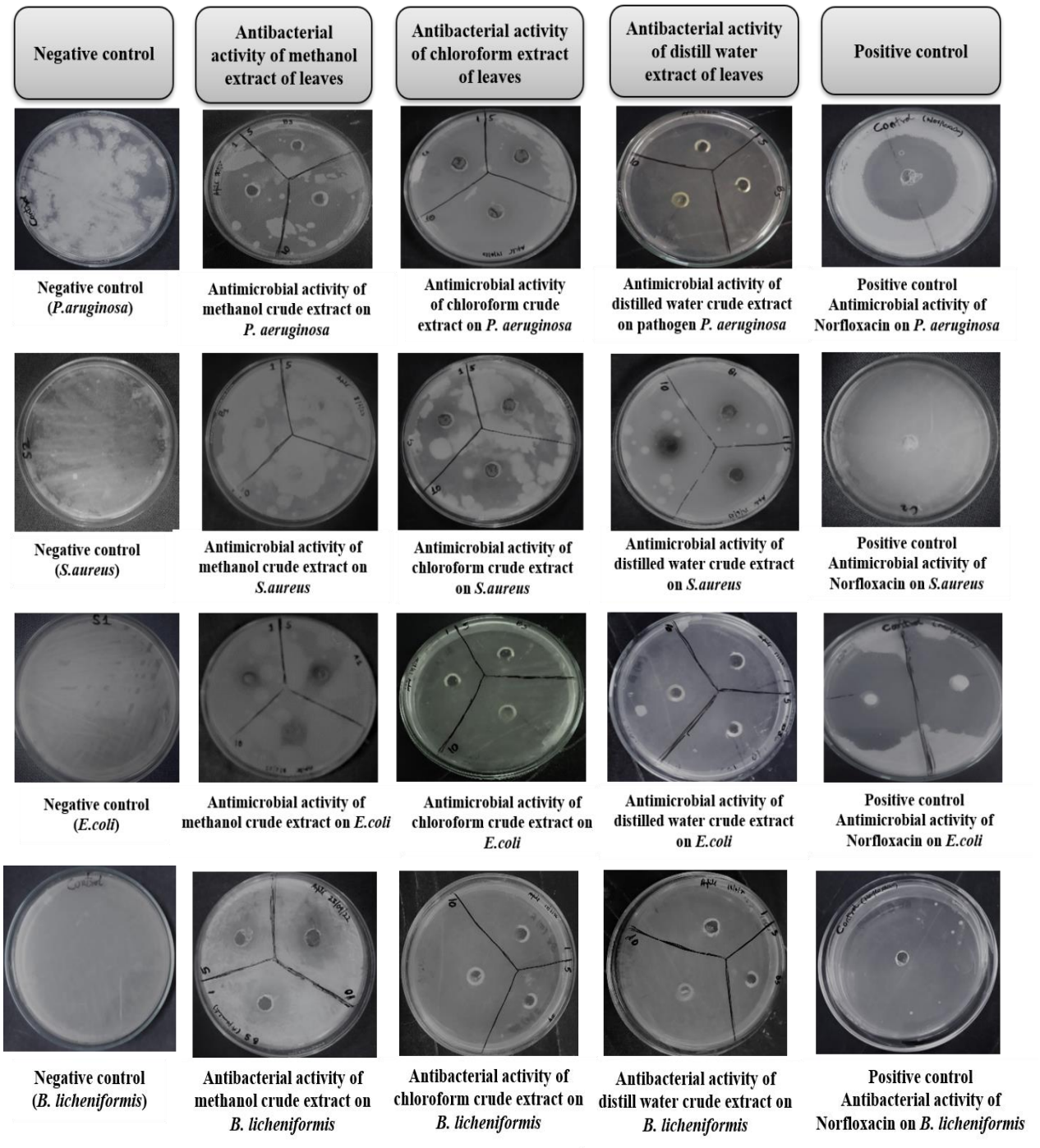

Fig. 2 Antibacterial activity of the crude extracts of leaves of *Andrographis paniculata* of different regions as well as the effect of antibiotic Norfloxacin on *P. aeruginosa*, *S. aureus*, *E. coli* and *B. licheniformis*. The negative control plates consist only pathogens.

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1943-1958 | ISSN:2251-6727

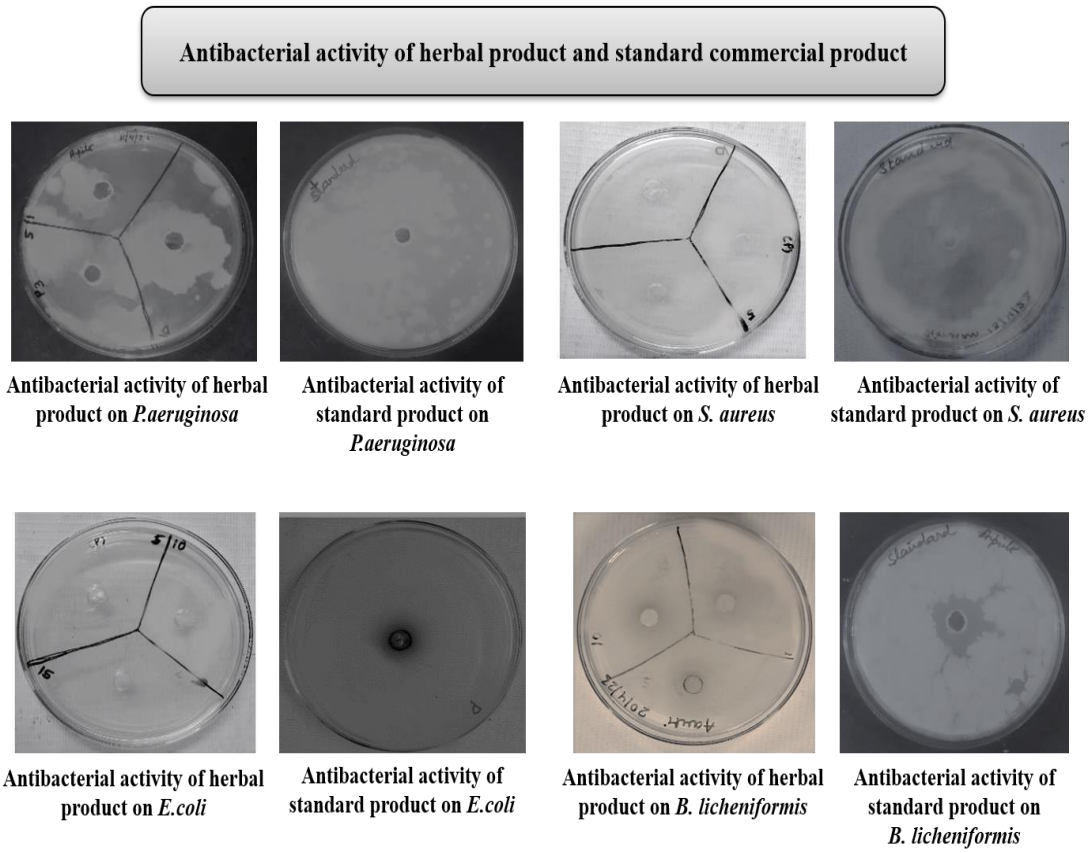

Fig. 3 Antibacterial activity of the herbal product and standard commercial product on P. aeruginosa, S. aureus, E. coli and B. licheniformis.

Table. 1 Antibacterial activities of the crude extracts of leaves of *Andrographis paniculata* and herbal intimate products as well as the effect of antibiotic Norfloxacin and standard commercial product on bacterial pathogens by Agar well diffusion where the bacteria have found to be as R=Resistant, *- = Sensitive, Zone of inhibition in mm.

UTI causing pathogens	Activity of crude extracts of leaves of *A. paniculata* at different concentrations in mg/ml									Activity of herbal intimate product at different concentrations in mg/ml			Positive control standard commercial product	Positive control Antibiotic (Norfloxacin)
	Methanol			Chloroform			Distill water							
	1	5	10	1	5	10	1	5	10	1	5	10		
P. aeruginosa	R	R	*-	*-	R	*-	*-	*-	*-	R	*-	R	R	*- 50 mm
S. aureus	R	R	R	R	R	*-	R	*-	R	*-	R	*-	*- 55mm	*-

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1943-1958 | ISSN:2251-6727

E. coli	**R**	**R**	**R**	***-**	***-**	***-**	***-**	***-**	***-**	**R**	**R**	***- 8mm**	***-**	***-**
B. licheniformis	**R**	***- 12mm**	***- 16mm**	***-**	***-**	***-**	***-**	***-**	***-**	***-**	**R**	***- 10mm**	***- 18mm**	***-**

Table 2. Minimum Inhibitory Concentration of crude extracts and formulated herbal product (soap) of *Andrographis paniculata* on bacterial pathogens.

UTI causing pathogens	**Minimum Inhibitory Concentration of crude extracts of leaves of *A. paniculata* at different concentrations in mg/ml**				
	Chloroform extract				
	1mg/ml	**2mg/ml**	**3mg/ml**	**4mg/ml**	**5mg/ml**
P. aeruginosa	0.055833± 0.002857	0.202467±0.00607	0.0492±0.015431	0.1887±0.007264	0.113633±0.0169
S. aureus	0.035767± 0.00317	0.03426± 0.00190	0.002133± 0.000289	0.0027±0.00017	0.0064±0.00017
E. coli	0.0072±0.00012	0.0130±0.00125	0.0466±0.00122	0.0174±0.00103	0.0066±0.00219
B. licheniformis	0.0073±0.00025	0.0119±0.00012	0.0459±0.00137	0.0181±0.00048	0.0251±0.00300
	Methanol extract				
	1mg/ml	**2mg/ml**	**3mg/ml**	**4mg/ml**	**5mg/ml**
P. aeruginosa	0.0066±0.0022	0.0174±0.0010	0.0456±0.0048	0.0137±0.0024	0.0072±0.0001
S. aureus	0.022433±0.008372	0.0476±0.013428	0.002133±0.000289	0.031167±0.039751	0.0063±0.0003
E. coli	0.0060±0.0031	0.0068±0.0019	0.0174±0.0009	0.0227±0.0048	0.0223±0.0025
B. licheniformis	0.035767± 0.00317	0.03426± 0.00190	0.002133± 0.000289	0.0027±0.00017	0.0064±0.00017
	Distill water extract				
	1mg/ml	**2mg/ml**	**3mg/ml**	**4mg/ml**	**5mg/ml**
P. aeruginosa	0.17183333±0.02250	0.1491333±0.03471085	0.081733333±0.051118033	0.1787±0.0062	0.113633±0.0169
S. aureus	0.0065±0.0009	0.0061±0.0009	0.0166±0.0004	0.0154±0.0002	0.0156±0.0004
E. coli	0.0454±0.0004	0.0489±0.0002	0.0364±0.0008	0.0168±0.0003	0.0118±0.0016

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 1943-1958 | ISSN:2251-6727

B. licheniformis	0.0600±0.0036	0.0514±0.0002	0.0635±0.0003	0.0439±0.0003	0.0291±0.0008
	Minimum Inhibitory Concentration of herbal intimate product at different concentrations in mg/ml				
	1mg/ml	**2mg/ml**	**3mg/ml**	**4mg/ml**	**5mg/ml**
P. aeruginosa	0.116823333±0.0931	0.01873± 0.00020	0.0627±0.048265826	0.08604±0.009664926	0.0829±0.00052915
S. aureus	0.0600±0.0036	0.0514±0.0002	0.0635±0.0003	0.0439±0.0003	0.0291±0.0008
E. coli	0.105166+0.0796	0.0858±0.09736	0.081733±0.05111	0.1887±0.0072	0.1136333±0.016
B. licheniformis	0.1855±0.0146	0.2477±0.0050	0.1859±0.0002	0.1746±0.0019	0.1486±0.0028

It was found that the viability was highest at 0.15 mg/ml with the value of 60%. The results showed that further reduction in drug dosage may lead in the improvement of cell viability.

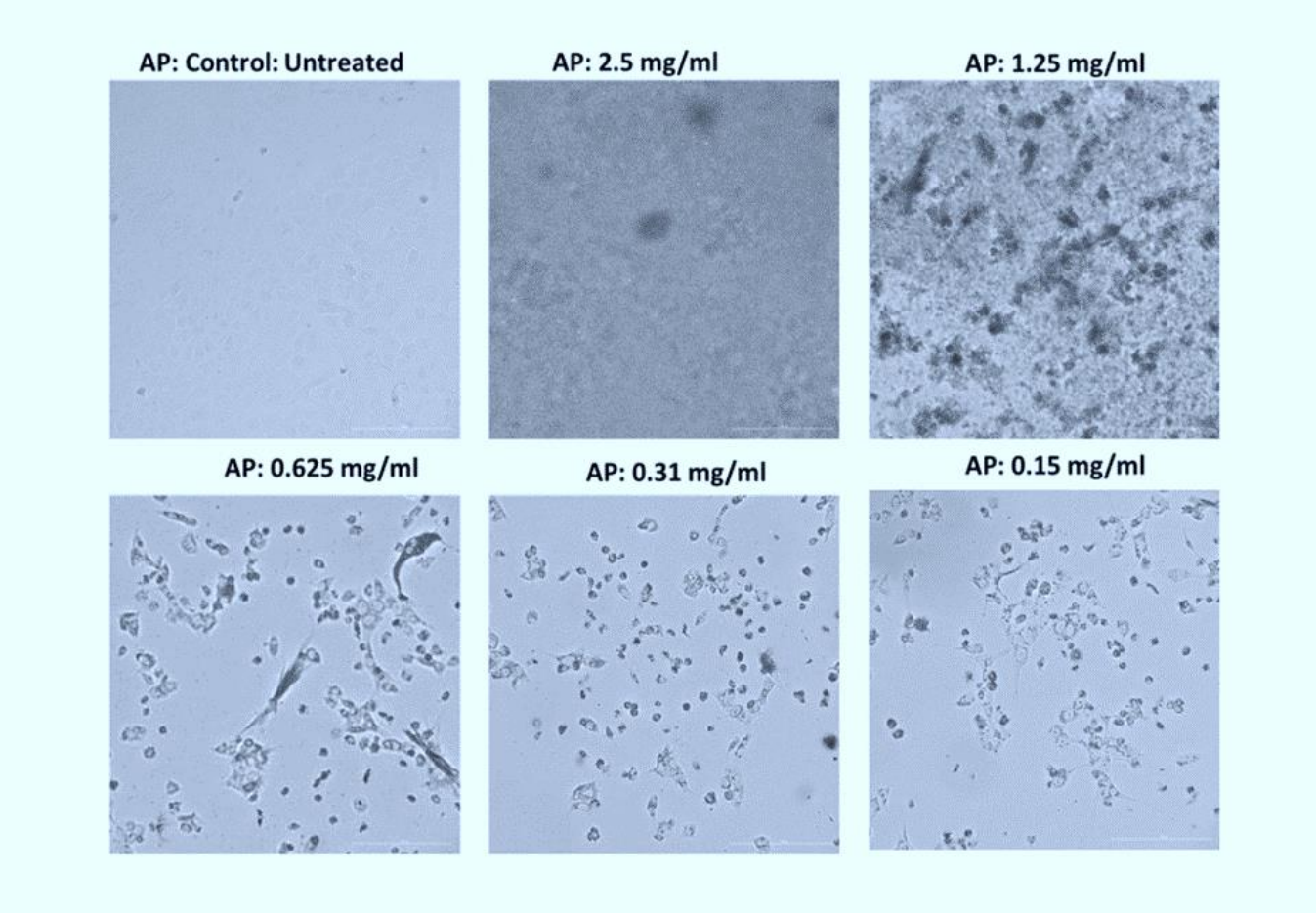

Fig. 4 Morphological changes in HaCat skin epithelial cells after 72 h treatment with different concentrations of herbal soap consisting the extracts of *A. paniculata*

Journal of Chemical Health Risks
www.jchr.org
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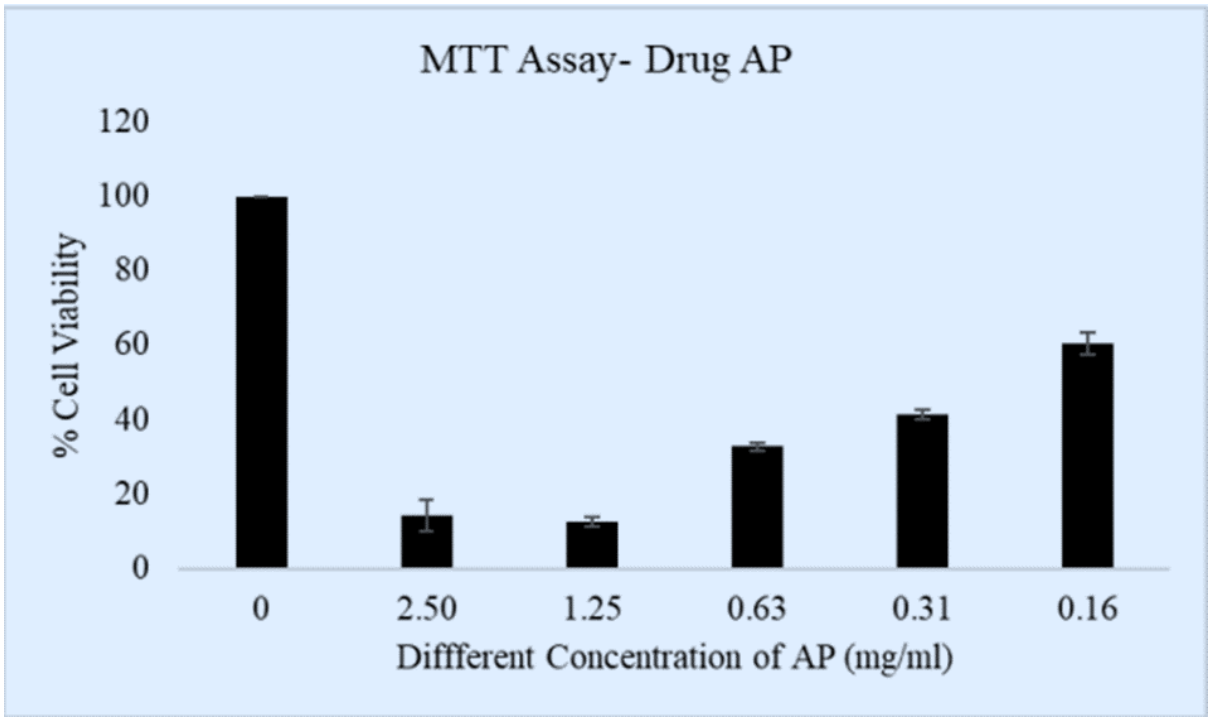

Fig. 5 Percentage viability of HaCat skin epithelial cell line after 72 hrs treatment of herbal soap (AP).

5. Discussion

Previous investigations have also reported the presence of certain phytochemicals in the plant. For instance, a study identified the presence of steroids, terpenoids, flavonoids, and phenolic compounds [25]. Similarly, a study reported the presence of alkaloids, carbohydrates, saponins, proteins, phytosterols, phenolic compounds, flavonoids, and glycosides in the ethanolic extract of A. paniculata leaves. Another study accomplished a preliminary investigation on the phytochemical screening of Andrographis paniculata seeds and confirmed the presence of tannins, flavonoids, saponins phenolic compounds, glycosides and terpenoids, in the methanol extract. Tannins, phenols, and terpenoids were found in chloroform, ethyl acetate, and methanol extracts, whereas flavonoids existed in both methanol and chloroform extracts [26]. A study determined the occurrence of phytocompounds such as phenols, saponins, tannins alkaloids, terpenoids, flavonoids, hydrolysable tannins, and glycosides in aqueous and alcoholic extracts of the whole plant of Andrographis paniculata [27-28]. Additionally, unrelated studies conducted and evaluated the phytochemicals and anti-psoriatic activity of the ethanolic extract of Momordica charantia leaves, and Rana and Kumar, 2022 investigated the antioxidative potential of propolis on Staphylococcus aureus infected BALB/c mice, highlighting its effectiveness as a free radical scavenger and potential therapeutic agent against staphylococcal infections [29-30]. The evaluation of the antioxidant properties of Kalmegh was done and discovered that A. paniculata, natural product showed 87 % higher scavenging potential than the leaf which was 86 % and stem showing 80.48 % [31]. In a study concurrent with another investigation, extracts from A. paniculata leaves (hexane, ethyl acetate, chloroform, and methanol) were evaluated for DPPH activity at concentrations ranging from 25 to 100 mg/ml. The results revealed a notable increase in inhibition of the DPPH radical with rising concentrations of the extracts [32].

Medicinal plants play a pivotal role as primary sources of ground breaking drugs, complementing the conventional pharmaceuticals. These therapeutic plants find extensive use in medicine, especially when dealing with drug-resistant bacterial infections. By harnessing the power of these natural remedies, we can address the challenge posed by antibiotic-resistant bacteria effectively. The widespread utilization of medicinal

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1943-1958 | ISSN:2251-6727

plants underscores their significance in providing alternative and innovative solutions to medical treatment. The potent antimicrobial behaviour has reported by various researchers earlier testified that 75% methanol crude extract of the leaves of A. paniculata was found to be vigorous counter to the pathogens M. tuberculosis, S. aureus and E. faecalis [33]. Similarly, it was reported that the water crude extracts of A. paniculata comprises probable antibacterial activity towards both gram positive and gram-negative bacteria. According to the results the aqueous extracts of A. paniculata showed maximum antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa [34]. Similarly, explored the effectuality of antibacterial activity of aqueous extract of A. paniculata which was further allied to the existence of andrographolide and arabinogalactan proteins [35]. An analysis discovered the antibacterial activity of several solvent extracts counter to E. coli E. aerogenes, P. vulgaris, S. aureus, Klebsiella sp P. aeruginosa where the ethanol extract showed widespread range of activity also exhibited the ultimate zone of inhibition against P. aeruginosa and S. aureus congruently [36]. Similarly, evaluation of the Minimum Inhibitory Concentration of methanol extract of Andrographis paniculata against several pathogens. The MIC value of 100µg/ml was found to be effective against Salmonella typhi, Vibrio cholera and Shigellaboydii however for Bacillus licheniformis and Staphylococcus aureus, MIC was found to be effectual at 10µg/ml which exhibited an active capacity of Andrographis paniculata [27]. Another efficient potential of MIC was testified, in this study the antibacterial activity of ethanol crude extract was detected through MIC in contradiction of both gram positive and gram-negative organisms, The values of MIC for P. aeruginosa and S. aureus were found to be 0.75 mg/ml and 1.0 mg/ml [36]. Analysis of the efficacy of Cassia tora plant against the vaginal microflora amid rural and urban populations. Results revealed that Cassia tora unveiled potent antimicrobial activity counter to vaginal microflora [37]. Similarly, another study investigated the comparative study of antimicrobial activity of seed oil of Fennel (Foeniculum vulgare) against bacteria and fungi. It was found that fennel oil was more efficient in opposing bacteria as compared to fungi [38]. Some researchers worked on the comparative study of clove oil against bacterial and fungal species. The antimicrobial activity of clove oil was potent against both the species [39]. Countless bioactive compounds and therapeutic properties of Swertia chirayita reviewed and conveyed [40]. Additional reports were likewise specified concerning the therapeutic facets of Tinospora cordifolia. The phytochemistry in addition numerous pharmacological properties of Tinospora cordifolia was thoroughly reviewed [41]. Investigation was done and reported the antibacterial activity of ethanol, aqueous and acetone extracts of Abutilon indicum, Boerhavia diffusa Andrographis paniculata Corriander sativum, Plantago ovata, Bacopa monnieri, Bauhinia variegata, Flacouratia ramontchi, Embelia tfgerium, Euphorbia ligularia, Zinziber officinale, Terminalia chebula, Azadirachta indica, Ocimum sanctum and Cinnamomum cassia determined counter to 33 UTI isolates. The acetone and ethanol extracts unveiled significant activity contrary to UTI pathogens [42]. Another study reviewed the therapeutic significance of Butea monosperma [43]. Some studies reported the computational studies on new Leishmanial drug targets against Quercetin. This study helps in the identification of targets and development of anti-Leishmanial drugs [44]. More studies on the assessment of cranberry bush on MCF-7 human breast cancer cells which revealed the therapeutic potential of Viburnum opulus L. (VO) powder extracts on human breast cancer and normal breast epithelial cell lines [45]. Investigation on the anticancer potential of Solanum lycopersicum L. extract in human lung epithelial cancer cells A549. reveal the phytochemical profile, free radical scavenging potential, and anticancer activity of Solanum lycopersicum L. leaf extract [46]. In a study conducted it was found that incorporating plant extracts into basic soap improved its efficacy against Gram-positive microorganisms. The basic soap exhibited minimum inhibitory concentration (MIC) values of 3.13 mg/ml, while the herbal soaps showed lower MIC values of 1.57 mg/ml, indicating superior or comparable activity to commercial soap [47]. Similarly, a research investigated the antimicrobial properties of formulated herbal soaps containing varying concentrations of plant extracts against E. coli, S. aureus, and P. aeruginosa. The results revealed that herbal soaps with higher concentrations of plant extract exhibited larger zones of inhibition [23].

According to the investigation the pH of herbal soap was estimated 7.9. Others studies are also reported such as evaluation of the pH of a soap prepared from A. indica

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1943-1958 | ISSN:2251-6727

seed oil was 10.434 [24]. Whereas Bhat et al., 2014 estimated the pH of a herbal soap formulated incorporating extracts of C. fistula, Milletia pinnata and Ficus religiosa was reported as 7.02 [48]. The foam retention capacity of the herbal soap was 5min as compared to other studies and observed that the foam retention of herbal soap prepared with C. fistula reported 6 minutes [23]. It was reported that polyherbal soap formulation prepared using coconut oil, castor oil, neem oil, mentha oil and rose petals extract demonstrated 10 minutes of foam retention time [49].

The results of MTT assay indicted that the cytotoxicity of the herbal soap consisting the extracts of A. paniculata was dose dependent and the cell viability can be improvised by the reducing the drug dosage. This is similar to the acute toxicity studies done on A. Africana investigating that the chloroform extract of the leaf was found to be more toxic as compared to the aqueous as well as ethanolic extracts [50]. Investigation reported that crude methanol extracts of leaf of Rhododendron were incubated with cultured epidermal keratinocytes (human HaCaT cell line) and epithelial cells of the intestinal mucosa (rat IEC6 cell line) for 24 hrs and confirmed for their potential of cytotoxicity [51]. Other studies examined the beneficial effect of A. brevipedunculata extract (ABE-M) on 2,4-dinitrochlorobenzene (DNCB)-induced AD [52]. Similarly, reported in-vivo studies in which they explored that the higher values of leaf extracts of Aspilia africana obtained were obtained for LD50 under oral administration of the experimentation performed which specified that oral administration of leaf extracts of Aspilia africana could be safe for both human and animal. The aqueous extract also appeared to be of comparatively lesser toxicity based on its higher value of LD50 [50]. Evaluation on the cytotoxic outcomes of acetone extract of Ficus crocata (Miq.) Mart. ex Miq. (F. crocata) leaves (Ace-EFc) on cervical cancer cells. Its protective effect on hydrogen peroxide (H2O2)-induced lipoperoxidation was also studied along with cytotoxicity in non-tumor HaCaT cells. Cell viability and lipoperoxidation were determined with MTT and 1-methyl-2-phenylindole assays. The results revealed that the metabolites of leaves of F. crocata possessed antioxidant as well as cytoprotective activity against oxidative damage [53]. Studies reported that Andrographis paniculata when used individually has been commonly perceived as safe in remedy [54]. Similar findings were also reported in which they studied that Aspilia africana plant is regarded as a harmless herb consisting low toxicity when used singly in therapy [55]. Bacterial contagion is one of the mainly stern worldwide wellbeing issues. The emergence of bacterial resistance to antibiotics has become a pressing health crisis, necessitating the development of novel herbal products with different modes of action to combat this problem. In this quest, plants have long been a promising source for new drug compounds due to their significant contributions to human health [56]. Incorporating plant extracts with well-established antimicrobial properties could prove highly beneficial for various therapeutic applications. Therefore, exploring plant-based solutions may offer valuable alternatives to address this urgent healthcare challenge.

5. Conclusion

The utilization of herbal plants presents a promising opportunity for the development of innovative therapeutic agents. To explore this potential, the first step involves investigating the biological activity of plant extracts through in vitro studies. In this framework, extracts from the leaves of Andrographis paniculata have demonstrated significant antimicrobial activity against both gram-positive and gram-negative bacteria responsible for causing various infections. The cytotoxicity of the formulated soap as herbal product through MTT assay has also turned out to be dose dependent which ultimately showcase the biocompatibility of the herbal product with living tissues. This research serves as a crucial link between traditional Ayurveda and Siddha practices and modern scientific exploration. The positive outcomes from this study offer a hopeful path for the future development of herbal products derived from Andrographis paniculata leaf extracts, with a specific focus on targeting infectious pathogens. The potential advantages of such products for humanity are vast and far-reaching. This integration of traditional knowledge and contemporary research opens new possibilities for herbal-based remedies to combat various ailments effectively.

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