Characterization and Antifungal Activity of *Andrographisp aniculata* Herbal Gel

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Andrographisp aniculata (AP), a herbal plant, is claimed to possess various pharmacological activities such as antifungal agents. The aim of the present study is to formulate and evaluate the gel containing ethanol extract from AP for its characterization and topical antifungal activity against superficial mycoses. Gelling agent used was 100% petroleum jelly. In the present study, the different concentration of the AP ethanol extracts were homogenized with petroleum jelly for the formulation of gel. The characteristics of formulations i.e. physical appearance, pH, microbial contamination and stability were examined. Furthermore, antifungal evaluation was done using food poisoning technique. The results of formulations characteristics showed that the gels were transparent, homogenous, and smooth feel application. Further stability test for four months showed the constant stability of 5.0% (w/w) AP gels, with the best skin pH application which ranging from 5.03±0.02 to 5.50±0.02. The results for microbial contamination were tested using pour plate method. There was no visible colony on the agar plates at all gel concentrations tested, indicating the safe and antimicrobial nature of the formulations. The *in vitro* antifungal activity studies showed that the formulation recorded inhibitory effect on the growth of all tested fungi;*Trichophyton mentagrophyte, T. rubrum, T. interdigitale, Microsporum fulvum, M. nanum*and *M. canis*. Significant mycelial inhibition was seen at all concentrations tested ($p<0.05$). Thus, the present study concludes that the formulated gel are safe and having efficient antifungal formulations for the topical delivery of theAPethanol extract.

Keywords:*Andrographisp aniculata*, antifungal agents, gel, stability

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1. Introduction:

AP is a herb used for superficial mycoses treatment. Its locally name is hempedubumi and it wastraditionally used to treat some diseases. In literature, the plant had shown its anti-typhoid, anti-fungal, antibacterial, anti-malarial, anti-hepatitic, and anti-inflammatory activities. The plant medicinal properties are attributed by the presence of diterpene andrographolide and neo andrographolide(Siripong*et al.*, 1992).

In order to treat superficial mycoses, AP was also used traditionally by topical application. These superficial infections include dermatophyt or ringworm infections. Dermatophyt are superficial infections of skin, usually known as ringworm, such as *Trichophyton* sp. and *Microsporum* sp. These genus are generally known as *tinea*. There are also the existences of mold which can bring out skin infections. The dermatophytes are fungi that invade keratinized tissues. The infection is commonly cutaneous and restricted to the non-living cornfield layers (Kenneth *et al*., 2010).

Dermatophyt infections generally infect human keratin such asskin, nail, and hair. They are commonly encountered fungal infections and often present as skin lesion, itchiness, nail damage and hair loss (Robin and Tony, 2015). Generally, antifungal drugs were a common practise in treating dermatophytosis. However, their indiscriminate use has led to higher rate of antifungal resistance among microorganisms (García-Sosa *et al.*, 2011).

Therefore, medicinal plants have served as a research resource for most antifungal drugs, at the same time serving as a basis for chemical research and discovery of new drugs. AP is a source of various agents of therapeutic value and also inspiredthe development and manufacture of synthetic agents of antifungal treatment.

2.0 Materials and methods:

2.1 Plant materials

The fresh plants of AP were cleaned, dried and crushed into powder. A hundred grams of the AP was soaked and blended in one litre ethanol. The filtrated extraction was evaporated using a rotary evaporator.

2.2 Preparation of AP gel

The AP ethanol extract was mixed in petroleum jelly (Vaseline, Malaysia) and stirred using homogenizer. The ingredients were mixed to obtain concentrations; 1.5% (w/w), 2.5% (w/w), and 5.0% (w/w).

2.3 Preparation of PDA-AP gel plate

The AP ethanol extract gel at concentrations 1.5% (w/w), 2.5% (w/w), and 5.0% (w/w) were poured into the melted Potato Dextrose Agar (PDA) to get 1.0% (w/v), 5.0%% (w/v), and 10.0% (w/v) concentrations. For control, the PDA was added with petroleum jelly (Vaseline, Malaysia) alone to get the concentrations 1.0% (w/v), 5.0% (w/v), and 10.0% (w/v). All procedures required aseptic handling.

2.4 Preparation of fungal isolates

The experiment was performed with seven types of fungal cultures;*T. mentagrophyte*(F2087/06), *T. rubrum*(F34517/08), and *M. canis*(F1018/09) [isolated from skin scrapping cultures]; and *T. interdigitale*(7A/6/13), *M. fulvum*(7B/6/09), *M. nanum*(7B/6/09), and *M. gypseum* (7A/2/06) [isolated from quality control programme]. All fungal stock cultures belong to the collection of Mycology Laboratory, Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia. The primary cultures were prepared by inoculating the fungi onto PDA plates. After the growth of fungi was established, the colonies were confirmed and identified using the tease mount technique.

2.5 *In vitro* antifungal activity of AP gel

The PDA medium prepared earlier was subjected to the fungal culturing of the following fungi; *T. mentagrophyte, T. rubrum, T. interdigitale, M. fulvum, M. nanum, M. gypseum* and *M. canis.* The culture technique was carried out using the food poisoning method described by Banso and Adeyemo (2007). The plate was incubated 35°C for seven days. Percentage of mycelial growth inhibition was calculated as follows;

% mycelial inhibition= $[(d_c - d_t)/d_c] \times 100$;

d_c = colony diameter in control, d_t = colony diameter in treatment.

2.6 Statistical Analysis

Analysis of data in 2.5 was performed using the Statistical Package for Social Science, version 12.0 software. The calculation was carried out using General Linear Model, that is, repeated measures analysis of variance (ANOVA) for each group. A value of 'p' of <0.05 was considered as significant.

2.6 Characterizations of AP gel

The AP ethanol extract gel was characterized for its physical quality which includes the stability study, physical appearance, pH, and microbiological analysis. The formulations were tested at concentrations 1.5% (w/w), 2.5% (w/w), and 5.0% (w/w).

2.6.1 Stability studies of AP gel

AP formulations were stored in a closed cap tube and put in the dark at room temperature for four months. After that, the formulations were evaluated for its' appearance, pH, and microbiological assay.

2.6.1.1 Physical appearance

AP formulations were inspected for its' physical appearance (Devedaet *al*., 2010).

2.6.1.2 Measurement of pH

One gram of formulation was dissolved in 100 ml warm distilled water and kept for two hours. After that, the pH reading was measured using pH meter.

2.6.1.3 Microbiological assessment

The fresh prepared formulations and 4-monhts kept formulations were tested for their sterility by pour plate method.

2.6.1.3.1 Pour plate method

One gram of AP ethanol extract gel at concentrations 1.5% (w/w), 2.5% (w/w), and 5.0% (w/w) were dissolved and mixed well in 100 ml of warm sterile distilled water. 1 mL of each formulation and PDA or nutrient agar (NA) was poured in the plate under aseptic conditions. After they was solidified well, they were incubated at 35˚C overnight (SaradhaJyothi and Rao, 2010). The plate was observed for the colony growth.

3.0 Results and discussion:

3.1 *In vitro* antifungal activity of AP gel

The tested AP gel exhibited different levels of antifungal activity (Table 1). Results indicated that the growth of *T. mentagrophyte* and *M. fulvum* (at 5% (v/v)) was significantly inhibited at 0.99% (v/v) AP gel concentration ($P<0.05$). In concentration of 1.96% (v/v) AP gel, the mycelial inhibition of *T. mentagrophyte*, *T. rubrum* and *M. gypseum* were significantly increased (at 5% (v/v)), while for *T. interdigitale*, *M. fulvum* and *M. canis*it significantly inhibited the growth at 5% and 10% (v/v) ($P<0.05$). At 7.41% (v/v) concentrations of AP gel, it significantly increased the mycelia inhibition for all fungi tested except for *T. rubrum*, *T.*

interdigitale and *M. canis* (P<0.05). *M. nanum* showed no significant difference of mycelial inhibiton compared to the control of all parameters tested. Thus, this study showed the best antifungal activity at 7.41% (v/v) AP gel.

This study revealed the scientific basis of the traditional usage of AP. These findings were also consistent with some previous reports, where the antibacterial effect of AP extract was demonstrated against skin infections stemming from bacterial strains (Sule *al.*, 2010)and antifungal potential in treating skin disease(Tapsell*et al.*, 2006).

The results obtained in the present study indicate that AP gel is active against the pathogenic fungi and has a broad spectrum activity. The mycelial inhibition data recorded alsoshowed that fungi tend to show wide differences in the degree of their susceptibility to antifungal agents. Therefore, the treatment of superficial mycoses using AP gel has a great future.

3.2 Stability studies of AP gel

The results of stability studies showed that AP gels were transparent, homogenous, and smooth. As the gel formulations percentage increased, the gel became dark greenish in colour (Table 2). Further stability test for four months showed the constant stability of the gels (Table 3). The pH values recorded for preliminary and stability studies of the gel formulations were in the range of 5.50±0.02 to 6.28±0.23, regarded as acceptable to avoid possible irritation upon the application to the skin because the pH of the skin is 5.5. The stability test carried out onto the gel formulations revealed that the gel contained 5.0% (w/w) AP extract showed a better stability than 2.5% and 1.5% (w/w) AP extract. The pH for 5.0% (w/w) gel formulations showed the best skin application ranging from 5.03±0.02 to 5.50±0.02.

This study indicated that the developed AP gel having good stability. In the pharmaceutical context, gels are used as lubricants and as carriers for drugs for their local effects and percutaneous absorption (Das *et al*., 2010). A study by Pandey *et al.*(2011) onto herbal gel indicated that the gel having carbopol showed good viscosity. The gel was also stable at room temperature.

Since AP gel showed the best performance in terms of antifungal effect and stability, it suggested the potential usage of AP gel for human application.

3.3 Microbiological assessment of formulations

The results for the microbial contamination of the AP gel are presented in Table 4. In this study, there was no visible colony on the NA and PDA plates at all gel concentrations tested.

These showed that AP extract was good formulated in gel form indicated the safe and antimicrobial nature of the formulations.

The results indicated that the AP sterility was quite stable after four months' time at room temperature. Although most of the traditional herbal preparations in the market present a large number of pathogenic bacteria, this study has shown that contradictory results are the result of the methods of the preparation and the equipment and materials used when getting the formulations ready. Another source of contaminants could also be the personnel that introduce the bacteria when handling the materials (Abba *et al*., 2009).

In this study, most of the processing was done using the aseptic technique such as mixing the cream and gel into the prepared AP extract. Besides that, AP extract obtained by ethanol extraction preferably inhibited the microbial growth due to the a*lcohol's antimicrobial* activityas its ability to denature proteins (McDonnell and Russell, 1999). On these bases, the AP gel formulation can probably be explored in the management of its anti dermatophytic agent and further commercialized into the market.

4.0 Conclusion:

From the above study, it can be concluded that AP can be used along with polymers in the form of gel to provide antifungal effect. Looking at the bright side of the antifungal activities of AP gel, its stability and sterility, it may be appropriate to suggest using AP in treating dermatophytosis, same as standard antifungal drug. At the same time, AP gel probably did not demonstrate fungal resistant effect, as compared to standard antifungal drug. Therefore, the treatment of dermatophytosis using AP gel has indeed a great future.

Table 1. Antifungal activity of AP gel on growth of fungi (percentage of mycelia growth inhibition) after seven days incubation using food poisoning technique

Fungi	PDA concentration (v/v)	Percentage of mycelial growth inhibiton (%) *A. paniculata* gel formulation concentration (v/v)		
		0.99%	1.96%	7.41%
T. mentagrophyte	1%	10.76	12.28	34.58
	5%	24.22*	35.48*	5.02*
	10%	38.11	36.36	46.71
T. rubrum	1%	22.71	26.75	60.92
	5%	8.65	22.18*	50.41*
	10%	14.31	64.6	78.75*
T. interdigitale	1%	2.64	12.82	18.88
	5%	17.77	32.75*	35.84*
	10%	26.01	31.46*	34.73*
M. fulvum	1%	1.78	2.76	45.44*
	5%	12.08*	12.52*	58.98*
	10%	7.46	21.37*	24.88*
M. nanum	1%	2.36	2.48	5.54
	5%	2.77	3.45	6.45
	10%	1.07	3.53	5.17
M. gypseum	1%	20.59	16.17	30.4
	5%	22.62	47.8*	62.87*
	10%	27.57	50.66	61.03
M. canis	1%	3.54	3.41	12.16
	5%	9.06	10.26*	22.09*
	10%	6.96	14.61*	15.17*

Notes: * denotes significant when value of $p < 0.05$

Table 2. Preliminary studies data of AP gel

Percentage of AP gel formulations (w/w)	Physical appearance				pH
	Physical appearance	Colour	Homogenity	Texture	
1.5%	Transparent	Green	Homogenous	Smooth	6.28±0.23
2.5%	Transparent	Dark green	Homogenous	Smooth	6.19±0.14
5.0%	Transparent	Dark green	Homogenous	Smooth	5.50±0.02

Table 3. Stability studies data (at room temperature) of AP gel (after four months)

Percentage of AP gel formulations (w/w)	Physical appearance				pH
	Physical appearance	Colour	Homogenity	Texture	
1.5%	Transparent	Green	Homogenous	Smooth	5.00±0.06
2.5%	Transparent	Dark green	Homogenous	Smooth	5.08±0.16
5.0%	Transparent	Dark green	Homogenous	Smooth	5.03±0.02

Table 4. Microbiological studies data of AP gel

Percentage of AP gel formulations (w/w)	Preliminary studies		Stability studies (after four months/RT)	
	NA	**PDA**	**NA**	**PDA**
1.5%	NG	NG	NG	NG
2.5%	NG	NG	NG	NG
5.0%	NG	NG	NG	NG

NA=Nutrient Agar; PDA=Potato Dextrose Agar; RT=Room temperature; NG=No Growth

Competing interests:

The authors declare that they have no competing interests.

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