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HPTLC Estimation and Antibacterial Effect of Methanolic Extract of *Andrographis Paniculata* Stem Under Accelerated Storage Condition

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ABSTRACT

Andrographis paniculata (Acanthaceace) commonly known as "kalmegh" and also called as "king of bitters" is used from ancient time. The main active constituents present in Andrographis paniculata is Andrographolide. The present study deals with comparative study of assay by HPTLC method and antibacterial activity for Andrographis paniculata extract at accelerated storage condition for a period of 6 months. The antibacterial activity of methanolic extract of dried stems of Andrographis paniculata was determined by broth dilution method against gram-positive bacterial strain (Staphylococcus aureus) and gram-negative bacterial strain (Escherichia coli). For HPTLC method, the active marker, andrographolide and extract was spotted on the plates precoated with silica gel 60 F_{254} and developed using chloroform: methanol(9:1v/v) as mobile phase. Densitometric analysis was carried out at 226 nm. The method showed high sensitivity with good linearity over the concentration range of 200-1000ng/spot. The peak for andrographolide was observed at Rf of 0.34 ±0.02. The aim of our study was to observe the effect of accelerated storage on assay of pure marker and marker in the extract. The analysis was carried out at 1,2,3,6 months study as per ICH guidelines for stability testing of drug at storage condition of 40ºC ± 2ºC/75% RH ± 5% RH.

Keywords: Andrographolide, *Andrographis paniculata* Antimicrobial activity, accelerated storage condition.

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INTRODUCTION

Medicinal plants are the major sources for the therapeutic remedies of various ailments, one such plant is *Andrographis Paniculata* (Acanthacae) which has been used for centuries in Chinese traditional medicines[1]. The plant is also known as the 'king of bitters' because it is extremely bitter in taste in every part of plant body. It is a perineal herb that is grown abundantly in countries like India, Pakistan, sri lanka etc. it is also one of the widely used herb in ayurvedic formulations[2]. It has antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic Effects. *Andrographis paniculata* or its constituents have been used to treat cases of leptospirosis, pulmonary tuberculosis (especially the exudative type), tuberculous meningitis, and acute pyelonephritis [3].structure of andrographplode is shown in figure 1.

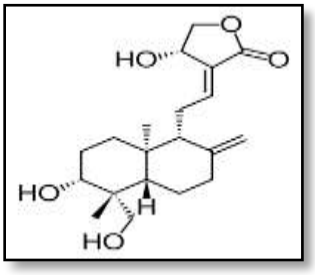

Figure 1: Structure of Andrographolide

A need has been realized to standardize the herbals in term of content of active constituents. Literature survey reveals several HPTLC [4-7] . HPLC [8-9], UV[10] methods for determination of andrographolide content from Andrographis paniculata. Some authors have reported antimicrobial activity for *Andrographis Paniculata* (stems) [11-13],Hence it was considered worthwhile to check if there is a direct relation between marker content in Methanolic extract of Andrographis paniculata and its antimicrobial activity even upon accelerated storage conditions for six months. In this study, the effect of storage conditions for 1,2,3,6 months at 40ºC ± 2ºC/75% RH ± 5% RH was evaluated. The stability of Andrographolide (marker) and *Andrographis Paniculata* (stems) extract was monitored using High Performance Thin Layer Chromatography (HPTLC) and Antibacterial activity study. The aim of this study was to check if marker assay of *Andrographis Paniculata* extract matches the antimicrobial action at accelerated storage condition.

MATERIALS AND METHOD

Chemicals and reagents

Andrographis Paniculata (stems) and andrographolide (marker) was purchased form Yucca

Enterprises, Mumbai, andrographolide (marker) was used as such, without any further purification *Andrographis Paniculata* (stems) were authenticated from Agharker Research Institute, Pune. Aluminum sheets precoated with silica gel (60 F_{254}, 20 cm × 20 cm with 250 µm layer thickness) were purchased from E-Merck, Darmstadt, Merck (Germany). Methanol (AR grade), Chloroform (AR grade), DMSO were purchased from S. D. fine chemical Laboratories, Mumbai.

Bacterial culture:

Bacterial cultures were purchased from National Chemical Laboratory, Pune.

gram-positive bacterial strain *Staphylococcus aureus* **(NCIM 2079)**

gram-negative bacterial strain *Escherichia coli***. (NCIM 2345)**

Chromatographic conditions and instrumentation

Chromatographic separation of drug was performed on Aluminum plates precoated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm layer thickness). Samples were applied on the plate as a band with 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Choloroform: Methanol (9: 1v/v). CAMAG twin trough glass chamber (10 cm × 10 cm) was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 ml of mobile phase was used per run, migration distance was 80 mm. Densitometric scanning was performed using Camag TLC scanner 3, operated by win CATS software (Version 1.4.3, Camag).

Preparation of extract:

4 gm of stem powder of *Andrographis Paniculata* was accurately weighed and dispersed in 100 ml of Methanol and was kept 24 hour for maceration. It was filtered through Whattman filter and the filtrate was evaporated at room temperature to obtain a solid mass of extract. The extractive value was noted, which was observed to be 4%.

HPTLC:

Preparation of standard (marker) solution:

Standard stock solution was prepared by dissolving 10 mg Andrographolide in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, working standard *Andrographolide* solution was prepared containing 100 µg/ml of Andrographolide for HPTLC method. Densitogram of marker is shown in figure: 2

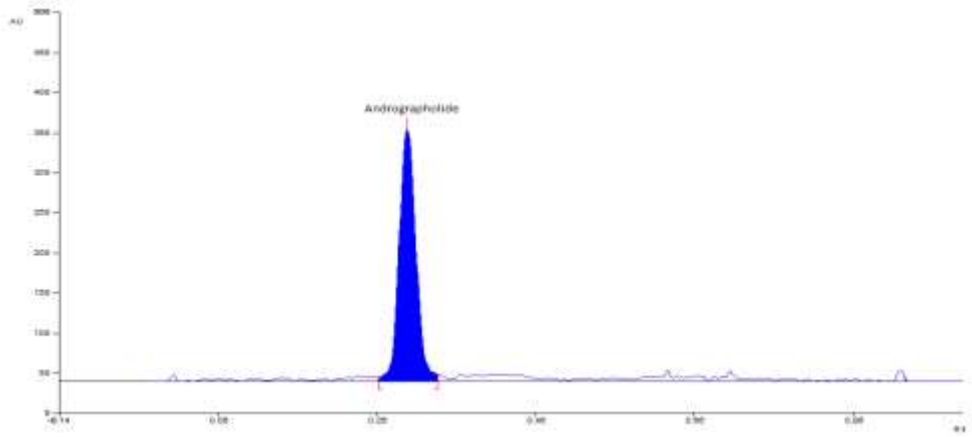

Figure 2: Densitogram of Standard andrographolide

Preparation of extract solution

10 mg of extract was dissolved in 10 ml of methanol to obtain concentration of 1000 µg/ml which was used for further studies

For Antimicrobial Study:

Preparation of extract solution

Methanolic extract solution prepared by dispersing 100mg extract in 10 ml DMSO.

A**NTIMICROBIAL ACTIVITY**

Broth Method

Broth method involves determination of minimum bactericidal concentration with using technique of variation in the colony count.

MIC Determination:

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after 18-24hrs. MIC was determined by Minimum bactericidal concentration. MIC determination was done at varying concentration range of 500-2000 µg/ml. The minimum concentration of the extracts that showed no detectable growth was 1800 µg/ml taken as the minimum inhibitory concentration.

RESULTS AND DISCUSSION

HPTLC

Andrographolide and methanolic extract was exposed to 40ºC± 2ºC, 75%±5 % RH for six months and the percent assay was noted for 1,2,3, and 6 months. It can be seen in table 1 and figure 3

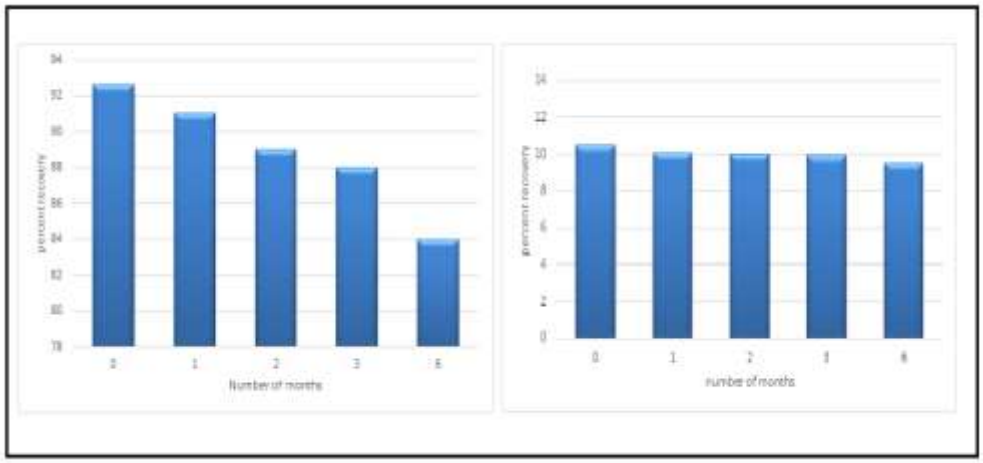

Figure 3: assay of andrographolide and methanolic extract of the stem over the period of six months

Table 1 % assay of Andrographolide in a) methanolic extract b) stability marker

Sr.no.	Month	%assay (extract) (A)	%assay (marker) (B)
1	Initial	10.51	92.6
2	First	10.06	91
3	Second	10.04	89
4	Third	9.97	88
5	Sixth	9.51	84

ANTIBACTERIAL ACTIVITY

Methanolic extract was exposed for 6 months at 40ºC± 2ºC, 75%±5 % RH as per ICH guidelines[14-16] and extract was withdrawn at 1,2,3,6 month to determine the antibacterial potential of the extract. Results revealed three log reduction in colonies count of extract, when used at 1800 µg/ml as can be seen in table 2 and figure 4

Table 2: Antibacterial Study of Methanolic Extract of Andrographis *Paniculata* (stems) After Exposure To 40^0c/75% RH For 1 To 6 Month.

Srno	month	Conc.(µg/ml)	Number of colonies (cfu/ml)			
			S.aureus **(Avg)**	***S.aureus***	***E.coli*** **(Avg)**	***E.coli***
1	Initial	1500	6.4×10^6	1.71×10^5	5.1×10^6	1.04×10^5
		1600		0.71×10^5		0.37×10^5
		1800		-		-
2	First	1500	6.9×10^6	1.74×10^5	4.9×10^6	1.08×10^5
		1600		0.70×10^5		0.41×10^5
		1800		-		-
3	Second	1500	7.3×10^6	1.77×10^5	5.2×10^6	1.11×10^5
		1600		0.74×10^5		0.44×10^5
		1800		-		-
4	Third	1500	6.6×10^6	1.78×10^5	5.4×10^6	1.14×10^5

		1600		0.74×10^5		0.44×10^5
		1800		-		-
5	sixth	1500	6.7×10^6	1.85×10^5	4.8×10^6	1.21×10^5
		1600		0.84×10^5		0.51×10^5
		1800		-		-

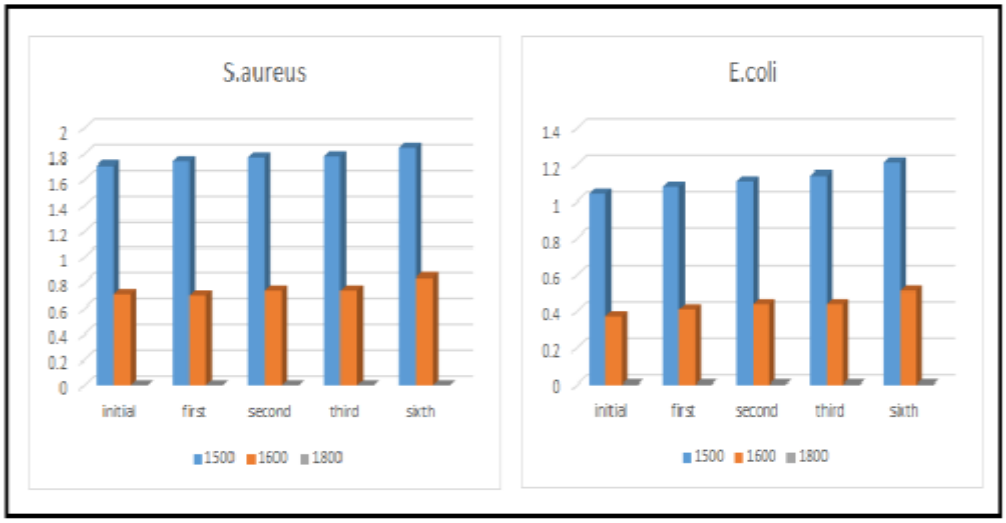

Figure 4: number of colonies methanolic extract of the stem over the period of six months

DISCUSSION AND CONCLUSION

For HPTLC after six month study, it was observed that there was substantial decrease in peak area of marker (Andrographolide), that was exposed to 40^0C/75% RH conditions and Andrographolide in methanolic extract.

One of the research paper indicated that *Anrographis paniculata* showed Antimicrobial activity against *S.aureus* . whereas no activity against *E.coli*; but our Experimental results show activity against both organism 1800µg/ml is the Minimum Bactericidal concentration of Methanolic exract as there was three log reduction in the colony count. After 6 month study it was observed that there was no change in antibacterial activity of the Methanolic extract of *Andrographis paniculata*. Thus concluding that Methanolic extract antimicrobial action was unchanged at 40ºC /75 % RH for six month, even though content of andrographolide reduced to 90 % of initial value.

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