Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(7); 965-969
doi: 10.25258/phyto.v9i07.11164

ISSN: 0975-4873

Research Article

Comparative HPTLC Estimation and Antibactrial Effect of Ellagic Acid, Gallic Acid and Ethanolic Extract of *Syzygium cumini* Seeds Under Accelerated Storage Condition

Dalavi N B[1*], Gawali V B[2], Bhalsing M D[3]

[1]*Department of pharmaceutical chemistry, AKCOP, Bodhegaon, Maharashtra, India.*
[2]*Department of Pharmacology, AKCOP Bodhegaon, Maharashtra, India.*
[3]*Department of Pharmaceutics, AKCOP Bodhegaon, Maharashtra, India.*

Received 12th May, 17; Revised 29th June, 17, Accepted 13th July, 17; Available Online 25th July, 2017

ABSTRACT

Syzygium cumini seed (L.) Skeels (myrtaceae) commonly known as "jamun" is widely used in Ayurveda. The main active constituents present in syzygium cumini seed is Ellagic and Ellagic acid. Syzygium cumini seed is official in Indian Ayurvedic pharmacopia. The comparative study of assay by HPTLC method and antibacterial activity was done for the Ellagic and Gallic acid and S.cumini extract at accelerated storage condition for a period of 6 months.The antibacterial activity of Ellagic acid, Gallic acid and ethanolic extract of dried seeds of s.cumini was determined by cup plate technique against gram-positive bacterial strain (Staphylococcus aureus) and gram-negative bacterial strain (Escherichia coli). For HPTLC method Ellagic acid, Gallic acid and extract was spotted on the plates precoated with silica gel 60 F_{254} and developed using toluene: ethyl acetate: formic acid, (6:6:1.5v/v/v) as mobile phase. Densitometry analysis was carried out at 271 nm. The method showed high sensitivity with good linearity over the concentration range of 200-1000ng/spot. The peak for Ellagic acid and Gallic acid were observed at Rf of 0.47 ±0.02 and 0.57±0.02 resp. The aim of our study was to observe the effect of accelerated storage on markers and 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. A decrease in antibacterial potential of the extract was observed with the simultaneous reduction in the % assay after a 6 month study. This method can be used for the quality control of the extract as well as markers.

Keywords: Ellagic acid (EA), Gallic acid (GA),Antimicrobial activity, *S. aureus, E.coli.*

INTRODUCTION

Plants have provided mankind with herbal remedies for many diseases for many centuries and even today. In India, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced such as Ayurveda, Unani and Sidha[1]. Ellagic acid (EA) and Gallic acid (GA) are found to be the active principles of *S.Cumini*. *S.Cumini* is official in Ayurvedic pharmacopeia[2]. which plays a vital role in the Ayurvedic system of medicine. The various phytoconstituents such as glycosides (jamboline), ellagic, gallic acid, tannins, fatty oil, steroids, flavonoids, triterpenes are present in *S.Cumini*.[3]. Its has pharmacological actions like antidiabetic[4], anti-inflammatory[5], antibacterial[6], antiallergic[7] and antioxidant[8]. The use of plant extracts and phytochemical, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency[9]. Literature survey reveals HPTLC profile of[10]. HPLC[11-13], HPTLC[14-15],RP-HPLC[16-18] antimicrobial activity of *syzygium cumini*[6-8], To the best of our knowledge, there is no any comparative method developed for determination of EA and GA and *S.cumini* seeds extract by HPTLC and antibacterial activity at accelerated storage condition. 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 EA, GA and *S.cumini* seeds extract was observed using High Performance Thin Layer Chromatography (HPTLC) and Antibacterial activity study. The aim of this study was to check if chemical assay of EA & GA match the antimicrobial action at accelerated storage condition.

MATERIALS AND METHOD

Chemicals and Reagents

EA and GA purchased form Yucca Enterprises, Mumbai, were used as such, without any further purification. *S.Cumini* seeds were purchased from local market & *S.Cumini* seeds 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 (HPLC grade), Ethanol (AR grade), Tolune (AR grade), Ethyl acetate (AR grade),

**Author for Correspondence: justnilam4141@gmail.com*

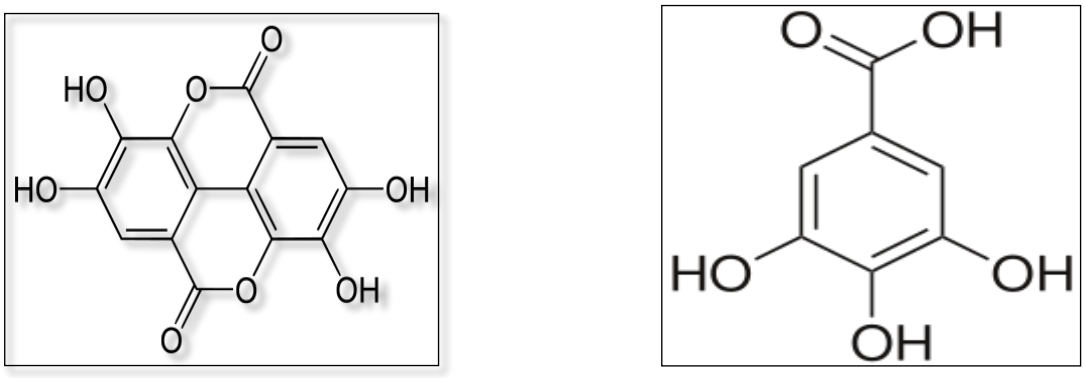

Figure 1: Structure of EA and GA.

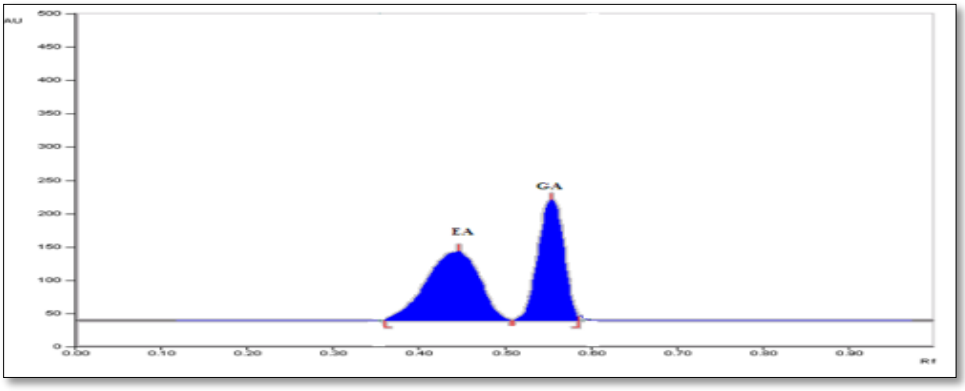

Figure 2: Densitogram of Standard EA and GA.

Table 1: Linearity & % Assay of EA.

Sr. No.	Sample	Conc (ng/ band)	Area (month)					%Assay (month)				
			0	1st	2nd	3rd	6th	0	1st	2nd	3rd	6th
1	mix	200	2073	2013	2020	2016	2013	-	-	-	-	-
2	mix	400	3510	3511	3525	3510	3504	-	-	-	-	-
3	mix	600	5517	5557	5567	5543	5520	-	-	-	-	-
4	mix	800	7202	7282	7245	7232	7210	-	-	-	-	-
5	mix	1000	8890	8898	8860	8880	8810	-	-	-	-	-
6	stability sample	400	3276	3276	3060	2590	2478	87.60	84.38	81.15	68.8	65.6
7	Extract	400	3340	3067	2691	1912	1520	0.734	0.661	0.567	0.393	0.303
8	Formulation	1000	1662	1401	1167	995	704	0.004	0.0039	0.003	0.0025	0.0016

Formic acid 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 2901)

gram-negative bacterial strain *Escherichia coli.* (NCIM 3321)

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 ethyl acetate: toluene :formic acid (6:6:1.2 v/v/v).10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 16 min saturation conditions and 13.2 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

A coarse powder of *Syzygium cumini* (L.) Skeels seed of was prepared and dried at 50^0C. The coarse powder extracted using ethanol in soxhlet apparatus. It was dried to obtain alcoholic extract yield was obtained 6%.

Preparation of standard solution

Table 2: Linearity & % Assay of GA.

Sr. No.	Sample	Conc (ng/ band)	Area (month)					%Assay (month)				
			0	1st	2nd	3rd	6th	0	1st	2nd	3rd	6th
1	mix	200	1887	1850	1868	1843	1859	-	-	-	-	-
2	mix	400	3872	3860	3877	3854	3865	-	-	-	-	-
3	mix	600	5647	5434	5465	5416	5434	-	-	-	-	-
4	mix	800	7474	7443	7456	7467	7465	-	-	-	-	-
5	mix	1000	9223	9239	9265	9254	9432	-	-	-	-	-
6	stability sample	400	3276	3676	3131	2567	1609	100	98.55	83.87	68.7	43.5
7	Extract	400	3698	3356	2787	2125	1580	0.807	0.725	0.596	0.454	0.342
8	Formulation	1000	8670	8056	7449	6580	4565	0.026	0.025	0.023	0.020	0.011

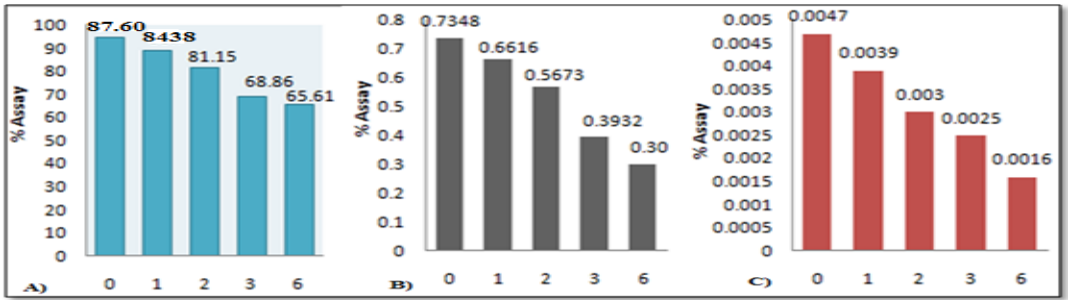

Figure 3: % Assay of EA in A) Stability sample B) Extract C) Formulation.

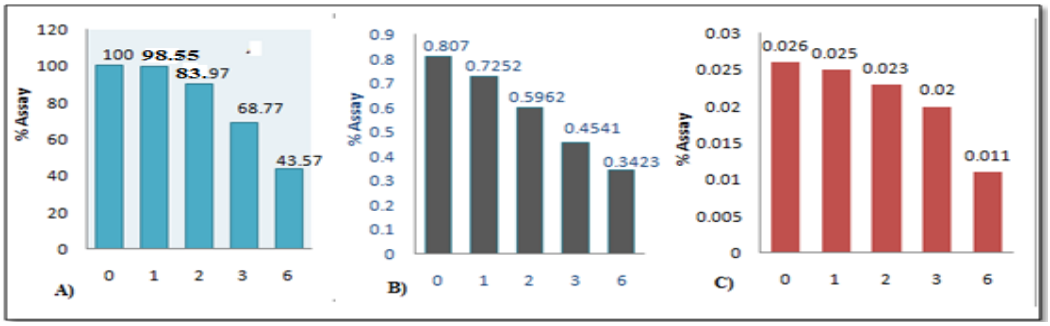

Figure 4:% Assay of GA in A) Stability sample B) Extract C) Formulation.

For Antimicrobial Study

Table 3 : Zone of inhibition.

Sample	Conc µg/ml	Zone of Inhibition(mm)									
		Staphylococus aureus					E. coli				
		Initial	1st	2nd	3rd	6th	Initial	1st	2nd	3rd	6th
extract	10000	18	16	14	12	10	16	15	13	11	9
GA	500	-	-	-	-	-	-	-	-	-	-
EA	500	14	14	14	14	14	12	12	12	12	12
-ve control	-	-	-	-	-	-	-	-	-	-	-
+ve control	1.56	10	10	10	10	10	10	10	10	10	10

For HPTLC

Standard stock solution was prepared separately by dissolving 10 mg EA and GA in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, working standard mixture of EA and GA solution was prepared containing 100 µg/ml of EA and GA for HPTLC method.

Standard stock solution of EA and GA were prepared separately by dissolving 10 mg of marker in 10 ml of DMSO to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 500µg/ml of GA and EA separately in DMSO for antimicrobial activity.

Preparation of Extract solution

Table 4: Statistical Comparison of HPTLC Assay and Antimicrobial Activity of extract at acclarated conditions.

Duration (Month)	% Assay of extract by HPTLC (X)	%assay of extract by Antimicrobial study		d = (y - x)		d ^{2}	
		S.aureus (Y)	*E.coli* (Y)	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*
initial	0.734	0.270	0.311	0.464	0.423	0.215	0.178
1st	0.661	0.235	0.284	0.426	0.377	0.181	0.127
2nd	0.567	0.228	0.236	0.339	0.331	0.114	0.109
3rd	0.393	0.166	0.184	0.227	0.209	0.051	0.043
6th	0.302	0.131	0.136	0.171	0.166	0.029	0.027
				∑d= 1.62	∑d= 1.50	∑d^2= 0.59 t=1.5	∑d^2= 0.48 t=1.7

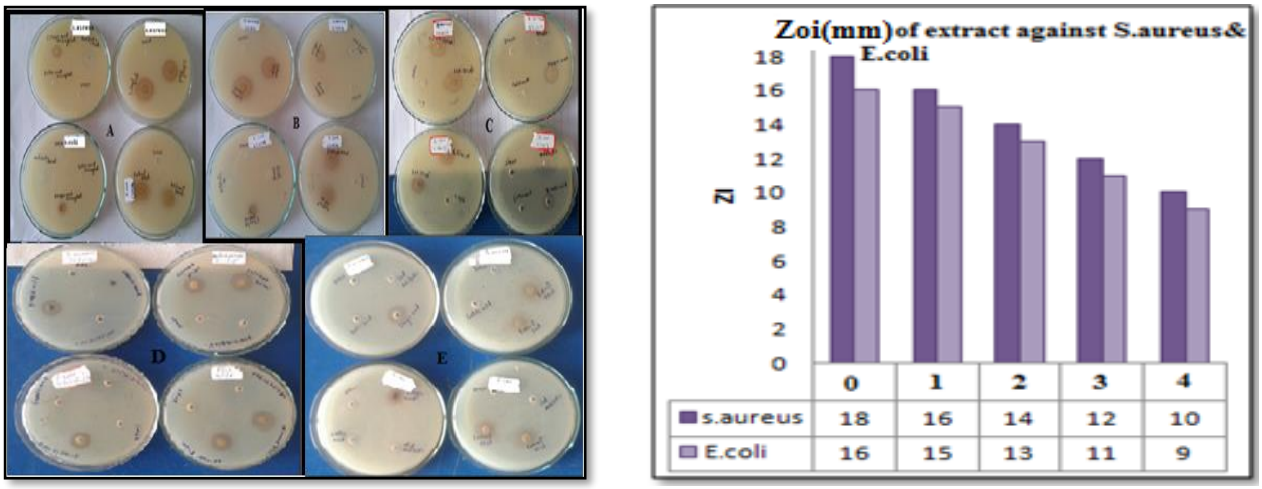

Figure 5: A) Antibacterial zone of inhibition shown by GA, EA and ethanolic extract A-Initial month study, B-I month study, C-II month study, D- III month study, E-VI month study,B) column chart of ZI shown by ethanolic extract.

Ethanolic extract solution prepared by dissolving 0.10gm extract in 10 ml DMSO.

Antimicrobial Activity

Cup Plate Method

The antimicrobial activity of extract was determined by CUP- plate method in Muller Hinton agar (oxoid/Diflo).All the glassware and the Petri plates were sterilized by autoclaving using 15 lbs pressure at 121^0C for 15 minutes. The antibacterial activity of the test compounds was assayed against *S.aureus* (gram+ve) and *E.coli* (gram-ve) by CUP-plate method. The medium was innoculated 10^6 cfu/ml of microorganism suspended in nutrient broth. Once the agar was solidified, it was punched with a six millimeters diameter wells. The wells were filled with 25µL of the test compounds of various concentrations and DMSO in wells was used as blank. The Petri dishes were incubated at 37 ± 1c^o for 24 hrs; the diameters of zone of inhibition (mm) surrounding each of the wells were recorded.

Zone of Inhibition (Zoi)

The antibacterial activity was screened by using CUP-plate method. All the bacterial cultures used were grown on nutrient agar medium at 37°C. Antibiotics such as Ofloxacin (1.56µg/ml) were used as positive control, while 100 % DMSO were used as negative controls. The diameter of the inhibitory zone was measured in mm using vernier calipers.

Determination of minimum inhibitory concentration:

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism. Standard antibiotic Ofloxacin and DMSO were placed as +ve and –ve controls resp . MIC was determined by Zoi. The minimum concentration of the markers, extracts that inhibited detectable growth was taken as the minimum inhibitory concentration.

RESULT

EA, GA, extract and formulation were kept in the Thermolab stability chamber for the 6 month at accelerated temperature and humidity conditions. During a storage period of 6 months a decrease in the peak area of EA and GA and substantial decrease in the % assay was observed The zone of inhibition was the largest when EA (300 µg/ml)and ethanolic extract (0.1gm) against *S.aureus*, it was least in case of *E.coli.* Gallic acid did not show any antibacterial activity against both *S.aureus* and *E.coli.* MIC of EA and extract was found to be 300 µg/ml, 0.1 gm resp.The HPTLC and antimicrobial study was compared

by applying statistical method for which matched paired t-Test was used.

Antibacterial Activity

The EA, GA and ethanolic extract was exposed for 6 months at 40ºC± 2ºC, 75%±5 % RH as per ICH guidelines, and the EA,GA and extract was withdrawn at 1,2,3,6 month to determine the antibacterial potential of the extract. Results revealed a decrease in zone of inhibition of extract which indicats a decrease in antibacterial potential of ethanolic extract of *S.cumini* seeds after a storage of 6 months, but antibacterial potential EA acid remain same.

CONCLUSION

This method gives a comparative idea about the effect of 6 months storage on the stability of the ethanolic extract & formulation. A decrease in % assay with subsequent decrease in antibacterial activity was observed. Quality control for herbal preparations or products, however is much more difficult than for synthetic drugs because of chemical ingredients complexity and any loss in particular chemical may result in loss of pharmacological action of that herb. Thus these methods may be used in phytopharmaceuticals formulation industry to monitor the stability of EA and GA content before taking up the production. It can serve as a Quality control parameter for herbal raw materials containing EA and GA.

ACKNOWLEDGEMENT

Authors are thankful to the Principal and the management of abasaheb kakade College of B.Pharmacy,Bodhegaon for providing the necessary facilities.

REFRENCES

1. Kunle M, Oluyemisi F. Review on *Syzygium Cumini* (L.) Skeels, International Journal Of Biodiversity And Conservation, 2012, Vol. 4(3), 101-112.
2. Ayurvedic Pharmacopoeia Of India. Part I, Vol-II.
3. Ayyanar M, Babu P. *Syzygium Cumini* (L.) Skeels: A Review Of Its Phytochemical Constituentsand Traditional Uses, Asian Pacific Journal Of Tropical Biomedicine, 2012, Vol 2(3), , 240-246.
4. Alam M, Rahman A. Evaluation Of Antidiabetic Phytochemicals In *Syzygium Cumini*(L.) Skeels, Journal Of Applied Pharmaceutical Science, Vol.2 (10), 2012, 94-98.
5. Muruganandan S, Srinivasans K. Anti-Inflammatory Activity Of *Syzygium cumini* Bark, Fitoterapia, 2001,Vol.7 (3), 369-375.
6. Kothari V, Seshadri S. Fractionation Of Antibacterial Extracts Of *Syzygium Cumini* (Myrtaceae) Seeds, Research In Biotechnology, 2011, Vol.2, 53-63.
7. Lima A, Ramos M. Pharmacological Study Of Anti-Allergic Activity Of *Syzygium Cumini* (L.) Skeels, Brazilian Journal Of Medical Biological Research,2 007,Vol 40, ,105-115.
8. Banerjee J, Narendhirakannan R. Phytochemical Analyses, Antibacterial, *In Vitro* Antioxidant And Cytotoxic Activities Of Ethanolic Extract Of *Syzygium Cumini* (L.) Seed Extract, International Journal Of Pharmaceutical Sciences And Research, 2011, Vol. 2(7), 1799-1806.
9. Muniappan Ayyanar, Syzygium Cumini (L.) Skeels: A Review Of Its Phytochemical Constituents And Traditional Uses, Asian Pacific Journal Of Tropical Biomedicine, 2012, 240-246.
10. Jadhav V, Kadam V. Development And Validation Of HPTLC Method For Determination Of 3-Hydroxy Androstane [16,17- C](6'methyl, 2'-1-Hydroxy – Isopropene-1-Yl) 4,5,6 H Pyran In Jambul Seed (*Syzygium Cumini*), International Journal Of Pharmtech Research, 2012,Vol.4, 1129-1135.
11. Gupta M, Sasmal S. HPLC Profiles Of Standard Phenolic Compounds Present In Medicinal Plants, International Journal Of Pharmacognosy And Phytochemical Research, 2012, Vol.4, 162-167.
12. Sawant L, Prabhakar B. Development And Validation Of HPLC Method For Quantification Of,Phytoconstituents In *Phyllanthus Emblic*,. Journal Of Chemical And Pharmaceutical Research, 2011, Vol 3(4), , 937-944.
13. Samee W, Vorarat U. Extraction Of Polyphenols And Anthocyanins From The Jambul *(Syzygium Cumini)* Fruit Peel, Thai Pharmaceutical And Health Science Journal. 2007,Vol. 2(2), , 131-137.
14. Syam M, Tamizhmani T. Pharmacognostic Studies Of The Leaves Of *Syzygium Cumini*, International Journal Of Research In Pharmaceutical And Biomedical Sciences, 2012,Vol. 3 (2) , 504-509.