International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

FORMULATION AND EVALUATION OF ANTIINFLAMMATORY ACTIVITY OF *SOLANUM PUBESCENS* WILD EXTRACTS GEL ON ALBINO WISTAR RATS

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ABSTRACT

The present study was aimed to prepared and characterize gel formulations of ethyl acetate and methanolic extracts of *Solanum pubescens* (Solanaceae) leaves using different polymers as gelling agents in various concentrations and also to evaluate anti-inflammatory activity of gel. For the study, polymers such as Carbopol 940 (0.5%w/v), Hydroxy Propyl Methyl Cellulose K_4M (2.5%w/v), Sodium Carboxy Methyl Cellulose (3.5%w/v) were selected for preparation of different gel formulations. The prepared gels were evaluated for physical appearance, pH, viscosity, extrudability, spreadability, anti inflammatory activity and also skin irritation to observe toxicity or side effects. At last the stability study performed to confirm the stability of final formulation. It was inferred from the results that gel formulation prepared by HPMC K_4M found to be best formulations among the prepared batches. The prepared gels were evaluated for anti inflammatory activity by carrageenan induced paw odema method in wistar rat model using Diclofenac Sodium as the reference anti-inflammatory drug. And HPMC K_4M formulation having 7.5%w/v ethyl acetate extract of *Solanum pubescens* (Solanaceae) leaves in gel shown significant inhibition in carrageenan induced paw odema.

Keywords: Anti-inflammatory, *Solanum pubescens*, Carrageenan, Diclofenac sodium

INTRODUCTION

The word inflammation, a defensive reaction to injury with classical signs of warmth, reddening, pain, swelling and loss of function, which is of a acute or chronic type [1] this inflammation is also observed in cancer [2], bowel syndrome, hepatic and Alzheimer's diseases. The characteristics of inflammation are humorous like reddening (visible), swelling (odema), soreness (pain) and corresponding histological changes. Nonsteroidal and steroidal drugs are generally used to treat inflammation. However, these drugs have side-effects like nausea, vomiting, etc [3]. *Solanum pubescens* (Solanaceae) are well known as Usti in Telugu and Kattusundai in Tamil (India). Previously reported compounds from the plant were flavonol-3-o-methyl esters [4]. Scientifically the plant used as Antilice [5] and Anthelmintic activity [6]. This plant is having folkloric claims used in inflammation and arthritis. In this work, we studied the anti-inflammatory activity of ethyl acetate and methanol leaf extract of *Solanum pubescens* in rats. The present investigation involves the preparation of three gel formulations of extract of *Solanum pubescens* followed by the evaluation for physical appearance, pH, Viscosity, extrudability, spreadability, for anti-inflammatory activity study, skin irritation to observe toxicity or side effects and the stability study.

MATERIALS AND METHODS

Plant: *Solanum pubescens* (Solanaceae) leaves were collected from the Gopalapuram, Chittor dist, Andhra

Pradesh in the month February 2011 and authenticated by the taxonomist, Dept of botany, Andhra University and the specimen voucher no AUCP/BGR/2011/S06 preserved in the Department.

Materials: Carbopol 940, HPMC K_4M, Sodium Carboxy Methylcellulose was purchased from S. D. fine-chem. Ltd., Mumbai, India. All other reagents and chemicals used were of analytical grade.

Animals: Adult albino wistar male rats weighing between 175-200gms were used for the study (supplied by B.N.Gosh and Co., Calcutta). The animals were divided into six groups of six animals each. The experimental protocol has been approved by the institutional animal ethics committee and by the Animal Regulatory Body of the Government (Reg.No:516/01/a/CPCSEA).

Extraction: The dried leaves as coarse powder were soxhelet extracted with ethyl acetate (EAE) and methanol (ME). The solvent from the extract were removed under reduced pressure and finally dried in desiccators.

Preparation of gels

***Preparation of gels Carbopol-940 gel*:** Accurately weighed quantity of Carbopol 940 was dispersed in water with constant stirring using mechanical stirrer at 1200 rpm for 30 min. Then Carbopol 940 was dispersed, the both extracts of 7.0% w/v, 7.5% w/v, 8.0% w/v were added in propylene glycol and preservatives were added and mixed well. The pH was then adjusted to neutral (pH-7) using triethanolamine and stirred slowly till a clear gel was obtained [7] and labeled as F_1, F_2, F_3 (For Ethyl acetate extract) and F_4, F_5, F_6 (For Methanolic Extract).(Table-1). The same method was followed for preparation of control sample without adding any *Solanum pubescens* leaves extract and labeled as C_1.

Hydroxy Propyl Methyl Cellulose (HPMC-K_4M) gel: Accurately weighed quantity of both extract was transferred to a beaker and dissolved in PEG into which preservatives were added. HPMC K_4M was made to disperse in distilled water then heated up to 80˚C - 90˚C with continuous stirring and it was allowed to cool. The 7.0% w/v, 7.5% w/v, 8.0% w/v extract loaded PEG solution were added to HPMC-K_4M preparation and stirred vigorously to mix in cold condition and water was added to make up the volume and stirred in mechanical stirrer well to get a uniform gel [8] and labeled as F_7, F_8, F_9 (For Ethyl acetate extract) and F_{10}, F_{11}, F_{12} (For Methanolic Extract) (Table 1). The same method was followed for preparation of control sample without adding any *Solanum pubescens* leaves extract and labeled as C_2.

Sodium Carboxy Methylcellulose gel: Accurately weighed quantity of Sodium Carboxy Methylcellulose was dispersed in $1/4^{th}$ of water with constant stirring using a mechanical stirrer at 2000 rpm for 30 min. The 7.0% w/v, 7.5% w/v, 8.0% w/v extracts was made to dissolve in propylene glycol and preservatives were added and mixed well with remaining water to get a homogenous gel and labeled as F_{13}, F_{14}, F_{15} (For Ethyl acetate extract) and F_{16}, F_{17}, F_{18} (For Methanolic Extract). (Table 1). The same method was followed for preparation of control sample without adding any *Solanum pubescens* leaves extract and labeled as C_3.

Evaluation of gel formulations [9]: Prepared gels formulations were evaluated for physical appearance, pH, viscosity, spreadability, extrudability.

Physical observation [10]: Transparency and Homogeneity [11] were observed (Table 2).

Determination of pH: The pH of the gel was determined using a calibrated pH meter [12].The readings were taken for average of 3 samples (Table 3).

Viscosity determination or Rheological studies: The resistance of a substance to flow is called viscosity. It was measured in Pascal seconds or poises. The rheological studies were determined using LV-2 spindle in a Brookefield Viscometer Model LVDV-E, USA. All measurements were performed in triplicate (Table 3).

Determination of Spreadability: The spreadability [13] of the gel formulations was determined 48h after preparation, by measuring the spreading diameter of 1g of the gel between two glass plates after 1min. The mass of the upper plate was standardized at 125 g. The spreadability was calculated by using the formula

$$S = m.\ l/t$$

Where, S is spreadability, m is the weight tied to the upper slide, l is the length of the glass slide, and t is the time taken. All measurements were performed in triplicate (n = 3) (Table 3).

Extrudability: The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed.

The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair) [14]. All measurements were performed in triplicate (Table 3).

Anti-inflammatory activity: Albino rats weighing between 175-200g were taken and divided into four groups containing six animals each group. The anti inflammatory activity was carried out by local application of *Solanum pubescens* (Solanaceae) leaves extracts gel formulation by to the carrageenan-induced paw odema in to Albino wistar rat model of either sex and the odema on paw was measured in terms of thickness by using Zeitlin's apparatus (unpublished) [15]. The gels were applied half an hour before injecting carrageenan to the plantar surface of the hind paw by gently rubbing fifty times with index finger and paw thickness was measured. The 0.1ml of 1% v/w of carrageenan was injected into the subplantar surface and paw volume was again measured at the end of 0h, 1h, 2h, 3h, 4h, 5h and 6h after application. Control group rats received only the gel base without drug by same mode of application. The paw thickness of each rat paws measured by using Zeitlin's apparatus (unpublished) before and at 1, 2, 3, 4, 5 and 6 hrs after carrageenan injection. Paw thickness was measured by subtracting the initial thickness from the obtained value at every hour after carrageenan injection. All measurements were performed in triplicate (Table 4).

The percentage inhibition of paw odema was calculated by using formula-

% increase in paw thickness = $(Y_t\text{-}Y_0/Y_0) \times 100$

Y_t = Paw thickness at time t (1, 2, 3, 4, 5 and 6 hr) after injection

Y_0 = Paw thickness at 0 hr (before injection)

Data were expressed in terms of mean values ± S.E.M.

Statistical analysis: All values were expressed as mean ± S.E.M. The differences were compared using one way analysis of variable (ANOVA) followed by Dunnett's t-test and un-paired students t-tests. P-values (<0.05) were considered statistically significant [16].

Skin irritation test: 0.5g of the herbal gel was used as the test substance and applied to an area of approximately 6 cm^2 of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a semi-occlusive dressing for the duration of 1 hour and gauze was removed. At the end of the exposure period, i.e., 1 hour, residual test substance was removed, without altering the existing response or integrity of the epidermis. Observations were recorded after removal of the patch. Control animals were prepared in the same manner and 0.5g of the gel base i.e., gel formulated using all ingredients except the herbal mixture was applied to the control animals and observations were made as similar to the test animals [17]. The gel was applied to the skin once a day for 7 days and observed for any sensitivity and the reaction if any was graded as [18]: A – No reaction, B – Slight patchy erythema, C – Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema (Table 5).

Stability study: All formulations containing mucoadhesive polymers were tested for stability under the actual condition of storage. Gels were stored in clean, dry, airtight moisture proof bottles, kept away from light. After 1 month of proper storage of the formulation in the ambient temperature and humidity the gel samples were withdrawn and evaluated for pH, viscosity, gelation temperature, swelling index, spreadability, mucoadhesive strength, and drug content and drug release study [19] (Table 6).

RESULTS AND DISCUSSIONS

The *Solanum pubescens* leaf extract gel was translucent in appearance and gave smooth feel on application which was maintained after tested stability study (Table 2, 3, 4 &5). pH were maintained throughout the study at 7.0 for all the formulations. The initial viscosities of developed gels were measured using Brookfield viscometer with spindle. Further stability test for one month has been carried out and it was found that viscosity of gel formulation contain Carbopol 934 varies from 4200-4215 mpa s, in case of HPMC K_4M and Sodium carboxy methyl cellulose it was found to be varied 4889-4892 mpa s and 4012-4015 mpa s respectively. Spreadability of all formulation varied from 17-25 g cm/sec, Extrudability of all the formulation is higher than 90%. So it can be said that spreadability and extrudability of all the formulation shows good acceptance properties. The ethyl acetate extract of leaves of *Solanum pubescens* at the doses of 7.0%w/v, 7.5% and 8.0%w/v in all the three types of different polymer content formulation produced time related, sustained and dose dependent significant reduction (P<0.05-0.001) of carrageenan induced inflammation of the rat hind paw (Table 4) but only in case of HPMC K_4M formulation contain 8%w/v ethyl acetate extract shows zone of inhibition more

than standard so it was not accepted. A methanolic extracts of leaves of *Solanum pubescens* at the doses 7.0%w/v, 7.5%w/v and 8.0%w/v were not able to produce significant reduction, where as doses of 8.0%w/v produced significant reduction ($P<0.01$) in the inflammation produced by carrageenan when compared to the percentage reduction observed in Diclofenac sodium (standard) treated groups (Table 4).From the observed values, the percentage of maximal paw odema produced during 6 hours was calculated for all the extracts of the plant. And results revealed gel containing HPMC K_4M formulation having 7.5%w/v ethyl acetate extract of *Solanum pubescens* (Solanaceae) leaves in gel formulation showed significant activity [65.15 ± 3.20 (Percentage inhibition of maximal paw odema during 6hr),76.35 ± 4.28 (Percentage inhibition of total AUC paw odema during 6hr)] in comparison with standard Diclofenac [65.15 ± 3.20 (Percentage inhibition of maximal paw odema during 6hr),76.35 ± 4.28 (Percentage inhibition of total AUC paw odema during 6hr)]. Skin irritation study result of Various Optimized EAE and ME Extract Loaded Carbopol-934, HPMC-K_4M, sodium carboxy methyl cellulose Gel Formulations shows that there is no irritation after 7 days of application.

And from stability study it was found that Character of Various Optimized EAE and ME Extract Loaded Carbopol-934, HPMC-K_4M, Sodium carboxy methyl cellulose Gel Formulations after One Month remain same or a little change. As Initial viscosity HPMC K_4M formulation having 7.5%w/v ethyl acetate extract of *Solanum pubescens* (Solanaceae) leaves in gel were 4891±55.45 mpa s, spreadability 24.35±0.56 gm cm/sec, extrudability 96.73 ± 0.005% and after stability study there were not much variation. The gel was non-irritant upon application on to the skin (Table 5).

CONCLUSION

Formulation F_8 with 2.5% HPMC K_4M and 7.5%w/v ethyl acetate extract of *Solanum pubescens* was the best formulation with significant anti-inflammatory activity; the formulated gels were evaluated for gross visual appearance, pH, viscosity, spreadability, extrudability, anti-inflammatory with lesser side effects can be expected from this formulation as the drug from natural source. We can conclude that industrial manufacturing of this product can be taken up after conducting clinical trials on human volunteers. The present study was conducted to evaluate the anti-inflammatory activity of *Solanum pubescens*, which is very new herbal drug that was firstly identified by us to get a berth in the group of anti-inflammatory herbal drugs. Further studies may reveal the exact mechanisms of action responsible for the anti-inflammatory activities of *Solanum pubescens*.

ACKNOWLEDGEMENT

The authors are thankful to the Head of Department of Pharmacy, Andhra University and all staff of Unity College of Pharmacy, A.P. India.

Table 1- Composition of Various Optimized EAE and ME Extract Loaded CARBOPOL-934 / HPMC-K_4M / SODIUM CARBOXYMETHYL CELLULOSE Gel Formulations

COMPOSITION (% W/W)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}	F_{16}	F_{17}	F_{18}
EXTRACT	7.0	7.5	8.0	7.0	7.5	8.0	7.0	7.5	8.0	7.0	7.5	8.0	7.0	7.5	8.0	7.0	7.5	8.0
CARBOPOL-940	0.5	0.5	0.5	0.5	0.5	0.5	-	-	-	-	-	-	-	-	-	-	-	-
HPMC-K4M	-	-	-	-	-	-	2.5	2.5	2.5	2.5	2.5	2.5	-	-	-	-	-	-
SODIUM-CMC	-	-	-	-	-	-	-	-	-	-	-	-	3.5	3.5	3.5	2.5	2.5	2.5
PEG	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
METHYL PARABEN	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
PROPYL PARABEN	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
GLYCEROL	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
TRIETHANOL AMINE	qs	qs	qs	qs	qs	qs	-	-	-	-	-	-	-	-	-	-	-	-
WATER	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs

Table 2- Physical observation of Various Optimized EAE and ME Extract Loaded CARBOPOL-934 / HPMC-K_4M / SODIUM CARBOXYMETHYL CELLULOSE Gel Formulations

Formulation Code	Physical observation
C_1	Translucent non greasy homogenous
F_1	Translucent non greasy homogenous
F_2	Translucent non greasy homogenous
F_3	Translucent non greasy homogenous
F_4	Translucent non greasy homogenous
F_5	Translucent non greasy homogenous
F_6	Translucent non greasy homogenous
C_2	Translucent non greasy homogenous
F_7	Translucent non greasy homogenous
F_8	Translucent non greasy homogenous
F_9	Translucent non greasy homogenous
F_{10}	Translucent non greasy homogenous
F_{11}	Translucent non greasy homogenous
F_{12}	Translucent non greasy homogenous
C_3	Translucent non greasy homogenous
F_{13}	Translucent non greasy homogenous
F_{14}	Translucent non greasy homogenous
F_{15}	Translucent non greasy homogenous
F_{16}	Translucent non greasy homogenous
F_{17}	Translucent non greasy homogenous
F_{18}	Translucent non greasy homogenous

Table 3- pH, Viscosity, Spreadability, Extrudability of Various Optimized EAE and ME Extract Loaded CARBOPOL-934 / HPMC-K_4M / SODIUM CARBOXYMETHYL CELLULOSE Gel Formulations

Formulation Code	pH	Viscosity(mpa s) mean±SD	Spreadability(g cm/sec) mean±SD	Extrudability amount in % mean±SD
C_1	7.0	4212±51.25	20.45±0.70	94.55 ± 0.005
F_1	7.0	4215±52.41	20.34±0.71	93.55 ± 0.003
F_2	7.0	4218±42.44	20.31±0.72	93.12 ± 0.006
F_3	7.0	4219±51.78	20.25±0.65	91.51 ± 0.008
F_4	7.0	4213±65.43	20.34±0.56	92.34 ± 0.06
F_5	7.0	4213±51.40	20.33±0.68	91.12 ± 0.003
F_6	7.0	4215±53.71	20.23±0.71	90.34 ± 0.008
C_2	7.0	4889±72.23	25.42±0.72	97.56 ± 0.009
F_7	7.0	4890±65.44	24.61±0.72	96.78± 0.013
F_8	7.0	4891±55.45	24.35±0.56	96.73 ± 0.005
F_9	7.0	4892±52.71	24.23±0.71	95.68 ± 0.010
F_{10}	7.0	4889±62.47	24.12±0.79	96.13 ± 0.008
F_{11}	7.0	4890±52.45	23.09±0.75	95.34 ± 0.004
F_{12}	7.0	4891±52.73	21.01±0.74	94.56 ± 0.011
C_3	7.0	4012±71.21	19.13±0.76	93.78 ± 0.012
F_{13}	7.0	4012±62.42	19.02±0.67	92.24 ± 0.010
F_{14}	7.0	4013±52.44	19.02±0.64	91.67 ± 0.015
F_{15}	7.0	4013±52.76	18.68±0.79	91.11 ± 0.006
F_{16}	7.0	4013±62.40	18.23±0.71	92.78 ± 0.006
F_{17}	7.0	4013±52.41	17.25±0.65	92.15 ± 0.008
F_{18}	7.0	4015±52.72	17.14±0.71	91.90 0.003

Table 4: Percentage inhibition of Carrageenan induced paw odema in rats by treatment with Various Optimized EAE and ME Extract Loaded CARBOPOL-934 / HPMC-K_4M / SODIUM CARBOXYMETHYL CELLULOSE Gel Formulations and Diclofenac sodium

Treatments	Percentage inhibition of maximal paw odema during (6hr)	Percentage inhibition of total AUC paw odema during (6hr)
Standard (Diclofenac)	68.22 ± 1.84***	80.38 ± 5.63***
C_1	0.0± 2.26	0.0± 4.59
F_1	47.31 ± 3.26**	59.23 ± 2.12**
F_2	63.42 ± 2.18***	74.21 ± 1.62***
F_3	64.15 ± 3.20***	73.35 ± 4.28***
F_4	35.68 ± 7.19*	46.26 ± 2.28*
F_5	41.28 ± 2.55**	54.05 ± 3.47**
F_6	56.20 ± 2.22***	63.24 ± 6.91***
C_2	0.0± 2.22	0.0± 4.61
F_7	63.42 ± 2.18***	74.21 ± 1.65***
F_8	65.15 ± 3.20***	76.35 ± 4.28***
F_9	69.52 ± 2.18***	81.21 ± 1.65***
F_{10}	34.68 ± 7.22*	45.26 ± 2.05*
F_{11}	46.28 ± 2.61**	58.05 ± 3.41**
F_{12}	53.20 ± 2.43***	65.24 ± 6.85***
C_3	0.0± 2.26	0.0± 4.53
F_{13}	42.29 ± 3.22**	55.24 ± 2.26**
F_{14}	62.42 ± 2.17***	72.21 ± 1.60***
F_{15}	64.25 ± 3.29***	77.35 ± 4.27***
F_{16}	32.69 ± 7.19*	41.21 ± 2.12*
F_{17}	42.22 ± 2.24**	52.09 ± 3.13**
F_{18}	50.21 ± 2.87***	61.18 ± 6.55***

Table 5 - Skin irritation study result of Various Optimized EAE and ME Extract Loaded CARBOPOL-934 / HPMC-K_4M / SODIUM CARBOXYMETHYL CELLULOSE Gel Formulations

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
C_1	A	A	A	A	A	A	A
F_1	A	A	A	A	A	A	A
F_2	A	A	A	A	A	A	A
F_3	A	A	A	A	A	A	A
F_4	A	A	A	A	A	A	A
F_5	A	A	A	A	A	A	A
F_6	A	A	A	A	A	A	A
C_2	A	A	A	A	A	A	A
F_7	A	A	A	A	A	A	A
F_8	A	A	A	A	A	A	A
F_9	A	A	A	A	A	A	A
F_{10}	A	A	A	A	A	A	A
F_{11}	A	A	A	A	A	A	A
F_{12}	A	A	A	A	A	A	A
C_3	A	A	A	A	A	A	A

F_{13}	A	A	A	A	A	A	A
F_{14}	A	A	A	A	A	A	A
F_{15}	A	A	A	A	A	A	A
F_{16}	A	A	A	A	A	A	A
F_{17}	A	A	A	A	A	A	A
F_{18}	A	A	A	A	A	A	A

Table 6-Character of Various Optimized EAE and ME Extract Loaded CARBOPOL-934 / HPMC-K_4M / SODIUM CARBOXYMETHYL CELLULOSE Gel Formulationsafter One Month

Formulation Code	pH	Viscosity(mpa s) mean±SD	Spreadability(g cm/sec) mean±SD	Extrudability amount in %mean±SD
C_1	7.0	4211±51.21	20.43±0.72	94.54 ± 0.002
F_1	7.0	4214±52.23	20.32±0.72	93.51 ± 0.003
F_2	7.0	4217±42.11	20.23±0.71	93.10 ± 0.004
F_3	7.0	4217±51.45	20.15±0.63	91.50 ± 0.006
F_4	7.0	4213±63.46	20.33±0.34	92.32 ± 0.006
F_5	7.0	4213±51.49	20.11±0.34	91.10 ± 0.003
F_6	7.0	4215±53.75	20.09±0.45	90.29 ± 0.007
C_2	7.0	4889±72.26	25.41±0.62	97.53 ± 0.011
F_7	7.0	4890±65.47	24.39±0.27	96.74 ± 0.012
F_8	7.0	4891±55.34	24.33±0.45	96.72 ± 0.007
F_9	7.0	4892±33.45	24.21±0.22	95.59 ± 0.013
F_{10}	7.0	4889±63.57	24.13±0.91	96.11 ± 0.004
F_{11}	7.0	4890±51.78	23.11±0.65	95.32 ± 0.006
F_{12}	7.0	4891±50.54	21.02±0.77	94.56 ± 0.014
C_3	7.0	4012±69.42	19.14±0.71	93.77 ± 0.011
F_{13}	7.0	4011±62.22	19.05±0.63	92.19 ± 0.012
F_{14}	7.0	4012±52.67	19.01±0.61	91.36 ± 0.016
F_{15}	7.0	4013±52.08	18.56±0.72	91.09 ± 0.009
F_{16}	7.0	4013±62.67	18.21±0.78	92.72 ± 0.003
F_{17}	7.0	4014±52.33	17.21±0.61	92.11 ± 0.009

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