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### RESEARCH ARTICLE

#### FORMULATION AND EVALUATION OF ANTIFUNGAL TOPICAL GEL OF *C. tora* SEED EXTRACT

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#### Abstract

**Purpose:** To formulate novel topical antifungal gel containing seed extract of *C. tora*. and to evaluate their antifungal potential.

**Method:** the gel was formulated by a cold mechanical method. Prepared gel was subjected to various evaluation parameters like pH, viscosity, spread ability, homogeneity and antifungal activity.

**Results:** Prepared topical gel of extract was pH range of  $6.25 \pm 0.05$  to  $6.45 \pm 0.15$ , viscosity range  $386 \pm 27.90$  to  $680 \pm 45.50$ cp, spread ability  $40 \pm 2.5$  to  $38 \pm 2$  mm and homogenous. Prepared topical gel was shown zone of inhibition ranges from  $13.5 \pm 0.5$  to  $23.5 \pm 0.5$  mm for *Candida albicans*.

**Conclusion:** Formulation F6 was shown better antifungal activity as compared to its other formulations. The prepared gel formulation was found useful for topical application due to its neutral pH, good spread ability, low viscosity and no irritation on human skin.

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#### Introduction:-

Nowadays fungal diseases has become very common due to widespread uses of chemotherapeutic agents, antiviral drugs and changing life style of human being. All these factors cause a lowering of immunity power of the person. Among various fungal infections, those affecting skin and mucous membrane are frequently occurring. Although they do not cause any serious problem to the person but it devitalize the quality of the life of an individual (Gary Garber, 2001). Related to AIDS disease, there is a strain of fungi, *Candida*, which normally lives on skin, but under weak immunity it proliferates rapidly and crates an overgrowth (Indumathy R et al. IJPSDR, 2011, 3(1), 29-31). In most of the cases it has been observed that infections are caused by a species called *Candida albicans*. Skin diseases caused by *Candida* fungus includes nail fungus, jock itch, vaginal infection, oral thrush, diaper rash (David TS, Microbiology, 1960, 830-835). Presently in market, medicines are there for the treatment of fungal diseases, but all of them have undesired side effects (Barret D, Biochem Biophy Acta, 2002, 1587, 224-233). Also it has been observed that *Candida* sp. has developed resistance to those drugs which are used for its eradication. So to find a new antifungal candidate, investigators are trying to explore herbal molecule that can be used for its treatment (Parekh J et al., Africa J Biotech., 2008, 7(23), 4349-4353). So far, researcher are turning their attention towards folklore medicine as the *Candida* sp., has not acquired resistance to them as well as these medicines are cheaper and easily available (Fabricant DS et al., Environ Health Perspec., 2001, 109, 69-75). Species *Cassia* belongs to one such plant that possesses very wide active constituents. The plant is traditionally well known for the treatment of conditions like hypertension, diabetes, skin disease, cough, pulmonary problems, and stomach problem etc. Interestingly the genus *Cassia* consists of 580 species of trees, herbs and shrubs. Among various spp. of *Cassia* important ones are *C. auriculata*, *C. fistula*, *C. italica*, *C. javanica*, *C. siamea*, *C. spectabilis*, *C. alata*, *C. nodosa*, *C. tora*, *C. sophera*, *C. nigricans*, *C. angustif*

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olia, c. renegera and c. australis. Since due to widespread incidence of skin disease among population the plant cassia was felt effective for undertaking the formulation development (Hooker JD, 1879). The present research was worked out to find the antifungal activity of seed extricate of C. tora in contradiction of Candida albicans.

### Material and Approach:-

#### Assortment and Authentication of plant part:

The seeds of cassia tora were assorted from Nawabganj, Gonda, India in winter season. The plant was authenticated by Mr. Amit Kumar Shukla, KIPM, Gorakhpur. The seeds were bared under shadow, finely grounded and kept in closed bottle. The plant material was evaluated for foreign organic matter, ash value, loss on drying, water soluble ash value etc. The reagents used while experiments were of LR quality.



Fig. 1:- Cassia tora flowering twig.



Fig. 2:- Cassia tora Seeds.

#### Extraction of seedlings:

The finely grounded seed of the shrub was subjected to extrication in Soxhlet apparatus to methanol by hot continuous percolation method. The collected extract was concentrated on rotary evaporator and was kept in vacuum dryer until used. Fifty gram of the desiccated extricate was diffused in 500 ml methanol to get final concentration of 10 mg/ml. Methanol extractive value was also calculated. The plant extract was also subjected to UV spectrophotometric analysis to obtain absorption maxima. Qualitative analysis was done to find out the presence of glycoside, alkaloids, tannins, saponin and steroids.



Fig. 3:- Extraction Process



Fig.4:- Plant Extract.

## Methods:-

### Preparedness of Formulating Topical Gel:

Different gel formulation was prepared by cold mechanical method as per the constitution given in table below. The gel was made by using ingredients like polymer carbopol 934, PG-400, Ethanol, EDTA, propyl paraben, methyl paraben, Triethanolamine and distilled water in as sufficient quantity. For preparing the gel, the dist. Water was taken into two beaker, in one beaker exact quantity of plant extract was dissolved and into it weighed quantity of PG-400 and ethanol was added and into another beaker, weighed amount of carbopol 934, EDTA, propyl paraben, methyl paraben was dissolved while stirring constantly with the help of magnetic stirrer due to which, in dispersion, there was no clump formation. Now, the two solutions were mixed together with stirring in between and homogenous dispersion was obtained. To this mixture, Triethanolamine was added dropwise to obtain the gel consistency (Kitawat S et al., 2015, Patel S et al., 2018).

**Table 1:-** formulation development of c.tora seed extracts topical gel.

s. no.	components	Formulations					
		F-1	F-2	F-3	F-4	F-5	F-6
1	Seed extract(gm)	2.0	2.0	2.0	2.0	2.0	2.0
2	Carbopol 934(gm)	0.5	1.0	1.5	0.5	1	1.5
3	PG-400 (ml)	3.75	3.75	5	5	6.25	6.25
4	Methyl paraben(mg)	0.2	0.2	0.2	0.2	0.2	0.2
5	Propyl paraben(mg)	0.02	0.02	0.02	0.02	0.02	0.02
6	EDTA(mg)	0.03	0.03	0.03	0.03	0.03	0.03
7	Triethanolamine	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
8	Water	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100

The same procedure was adopted for preparing Fluconazole gel as standard drug.

### Grouping and Curing of Culture:

The colony of *Candida albicans* for investigation was procured from microbial department of Dr. RMLAU, Faizabad, and was safeguarded in Sabouraud's Dextrose Agar medium. The constitution of the SDA media and cell culture requirement is given below-

**Table 2:-** Constitution of SDA medium.

S. No.	Ingredients	Quantity taken (g/L)
1	Dextrose (Glucose)	40 g
2	Peptone	10 g
3	Agar	18 g
4	Distilled Water	1000 ml
5	pH	5.6

**Table 3:-** Culture requirement.

S. No.	Requirement	Ambient condition
1	Growth condition	Aerobic environment
2	Temperature	25-37 <sup>0</sup> C
3	Incubation time	5-7 days
4	Subculture period	28 days

### Calibration of Culture:

The culture of the test organism was normalized by spectrophotometry using McFarland turbidity standard (Baucer AW, Amr. J. of Clin. Path., 1966, 45(4), 493-496). To prepare the inoculum suspension, the test organism was first of all grown on SDA medium for 48 hrs. and then picked 6 colonies of 1.5 mm diameter and suspended in 5 ml 0.85% NaCl solution. The turbidity produced by this inoculum suspension was measured at 530 nm and was matched to turbidity produced by 0.5 McFarland turbidity standards. The inoculum suspension produced so

contained  $1.5 \times 10^8$  cells/ml. It was further modified with the liquid media to produce an inoculum suspension containing  $1.5 \times 10^6$  cells per ml (Barry Al., 1976).

**Preparation of Fluconazole:**

Fluconazole was taken as standard drug and 0.1 g of it was dissolved in 100 ml of DMSO (dimethyl sulfoxide) to obtain the ultimate concentration of 10 mg/ml.

**Assessment of Antifungal activity:**

The antifungal efficacy of Methanolic plant extract was evaluated by Agar cup bioassay method.

**Preparation of plates for inoculation:**

The petri plates used for inoculation purpose were made aseptic using oven at  $160^\circ \text{C}$  for 1-1/2 hr. The plates were filled with molten SDA (20 ml) aseptically in laminar air flow. After 30 min, keeping the plates at room temperature, the plates were inoculated with another layer of 5 ml of molten SDA containing 0.05 ml of normal cell lines of candida albicans. The hole was made in each agar plate with the help of cork borer no. 4.

**Determination of zone of inhibition:**

The required quantity of the gel was transferred into the cavities of petri plates, these plates were then refrigerated for 1h for pre incubation diffusion. After refrigeration the plates were normalized at room temperature and then incubated at  $37 \pm 1^\circ \text{C}$  for 3 days. The same experiment was carried out with standard drug Fluconazole. For accuracy of the result, the experiment had been conducted threefold and median values for zone of inhibition were calculated (Nascimento et al., 2000).

**In-vitro Drug Diffusion Study:**

For deducing this parameter for all gel formulations, the Franz-Diffusion cell apparatus was used. For making study, egg-membrane was fastened in between the donor and receiver compartment of the apparatus. The receptor compartment was maintained at a temperature of  $37 \pm 1^\circ \text{C}$  and was filled with 10.0 ml of phosphate buffer ph 6.8. For testing, 0.1 g of gel formulation was placed over egg-membrane and solution of phosphate buffer ph 6.8 in the receptor compartment with stirring at 50 rpm. Then the sample was withdrawn at regular time interval of 0, 1, 2, 3, 4, 5 and 6 hrs. and diluted with 10.0 ml of blank solution and sink condition was maintained.. Diffusion study of formulation was carried out in triplicate and average value  $\pm$  standard deviation was calculated.

**Release Kinetics:**

In order to predict the release behavior of functional component of the jelly formulation, obtained data were put in various models of mathematics. Kinetics of Zero order does not depend on concentration term while kinetics of First order depends on concentration, in which case the release of drug either follow bulging, desedimentation or plainly diffusion. To validate the obtained data, Higuchi model as well as Korsmeyers peppas model was utilized to confirm the mechanism of reaction (Martin, 1994).

**Evaluation of Designed Topical Jelly:****Determination of pH:**

To determine the pH of gel formulation 1% aqueous solution was made and stored for 1h. The pH was determined using digital pH meter. For accuracy the determination was done in triplicate and average value  $\pm$  standard deviation was calculated (Shah K, et al., 2012).

**Determination of Viscosity:**

The viscosity of the gel formulation was measured by Brookfield Digital viscometer. 5g of the gel sample was taken and placed in the sample holder of the viscometer and allowed to settle for 10 min and the viscosity measured at 20 rpm and temperature  $25 \pm 1^\circ \text{C}$  for 20 min. Viscosity was noted in centipoise and the reading was noted in triplicate (Ramchandani U et al., 2013).

**Determination of Spreadability:**

To determine this parameter Parallel Plate method was used with the help of "Wooden block" and "Glass" slide apparatus. For determination, two glass slides were used, one slide was put on the wooden block (ground slide) and 1.0 g of the sample was placed on it, then it was sandwiched by top slide. A pressure of 1.0 g was applied on the top slide for 10 min, to escape any air bubble and to obtain a homogeneous covering of the gel. The excess of the gel

oozing out from the side of the slides were scraped off with the help of knife. The top slide was then subjected to a pulling pressure of 2.0 g and was pulled to cover a distance of 7.5 cm. The time taken to cover this distance was noted and spreadability was calculated using the formula (Gandhi K et al., 2018).

Spreadability = wt. tide to top plate (g) X breadth (cm) of the glass pad / period in (sec) to cover the distance.

#### Homogeneity:

To check this parameter, all gel formulation was taken in suitable container and allowed to settle. The homogeneity was tested by visual inspection (Nawaz A et al., 2013, Ubaid M et al., 2016).

#### Determination of Functional constituent of Gel Manufactured (net content):

To determine the net content, 1g of gel formulation was taken in 50 ml volumetric flask and volume was made up to the mark by using methanol and trembled appropriately to solubilize the constituent in alcohol. Using what Mann filter paper, the solution was filtered and then 0.1 ml of the filtrate was taken in a beaker and mixed with 10 ml of the alcohol. The content of functional constituent were measured using spectrophotometer at 266 nm (Nandgude et al., 2008).

#### Stability studies of the Gel Formulation:

The formulated gel was subjected to stability studies as per ICH guidelines for a short period of time (3 months) in a stability chamber. The qualified herbal gel manufactured measuring 2% was filled to a humidity cabin (Floor standing model 3 units in one with individual temperature + humidity controller 300 X 300 X 300 mm, 15-60<sup>0</sup> C,) at 25°C ± 3°C/55% RH ± 5% RH, 30°C ± 1°C/55% RH ± 4% RH and 35°C ± 5°C/70% RH ± 4% RH. The aliquots were pipetted out at an interval of zeroth, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> months as well as valuated for pH, viscosity, spread ability and net content,

### Result & Discussion:-

#### Evaluation of plant material & plant extract:

The results attained are summarized below:

**Table 4:-** Preliminary evaluation of c. tora seed powder.

S.No.	Test	Result
1	Foreign matter	1.15 ± 0.10
2	Moisture content	8.00 ± 0.20
3	Total Ash value	7.20 ± 0.10
4	Water soluble ash value	3.25 ± 0.02
5	Acid insoluble ash value	1.05 ± 0.10
6	Methanol soluble extractive value	9.75 ± 0.25

**Table 5:-** Evaluation of c. tora Methanolic extract.

S.No.	Test	Result
1	Color	Dark coffee
2	Odor	Agreeable
3	% yield	10.35
4	Appearance	sticky
5	pH	5.7
6	Solubility	Water & methanol

#### Evaluation of various batches of Formulation:

Six different batches of formulation were made using 2.0 gm of methanol extract of seeds of C. tora and varying concentrations of carbopol 934 (0.5,1.0,1.5 g) and PG-400 (3.25,5.00,6.25 g). Carbopol 934 was used as gelling polymer because it is biodegradable, biocompatible, bioadhesive and nonabsorbable to the skin. Carbopol 934 has more gelling property in comparison to other carbomers (Blonco-Flonte et al., 1996). The quantity of polymer was optimized after making the herbal gel with different concentrations and the gel having 1.5 g was found to be suitable with the requirements of gel formulation. Propylene Glycol was used as permeation enhancer as it causes no erosion to human skin (Panigrahi, et al., 2006). Triethanolamine was used to adjust the pH of the gel

**Table 6:-** Evaluation of various batches of Formulation.

Parameters	F1	F2	F3	F4	F5	F6
pH	6.85±0.18	6.15±0.35	6.40±0.20	6.55±0.35	7.03±0.10	6.45±0.15
Viscosity	386±27.90	480±41.42	603±29.43	645±40.20	620±35.49	680±45.50
Homogeneity	++	+	++	++	++	+++
Spreadability (mm)	37±0.20	39.5±0.15	38±0.30	37.8±0.22	37.5±0.00	38±2.0
% Drug content	95.20 ± 0.30	94.76 ± 1.05	95.10 ± 0.85	95.55 ± 0.90	94.38 ± 0.60	97.40 ± 0.65

(\*Here +specifies fair, ++indicates good, +++ excellent)

The results of various parameters are given in the table. The pH values lies in the pH range which is comparable to the normal pH of the skin. The pH values of formulated gel ranges from 6.85 to 6.45 which lies in normal range. The measurement of viscosity shows that formulated gel was of low viscosity that satisfies the ease of application on skin. The viscosity of the gel was adjusted by the addition of a small quantity of Triethanolamine. The viscosity of F6 formulation which contained 0.5 gm of carbopol 934 yield satisfactory viscous gel consistency. The rheological study was also conducted on prepared gel formulation. The observed result shows that all gel formulation showed decrease in viscosity with increase in stress and makes them better for spreading on skin. In the glass plate method, the spread ability ranges from 37 to 38 gcm/s. All prepared gel formulation showed good homogeneity with an absence of lumps.

#### Drug content:

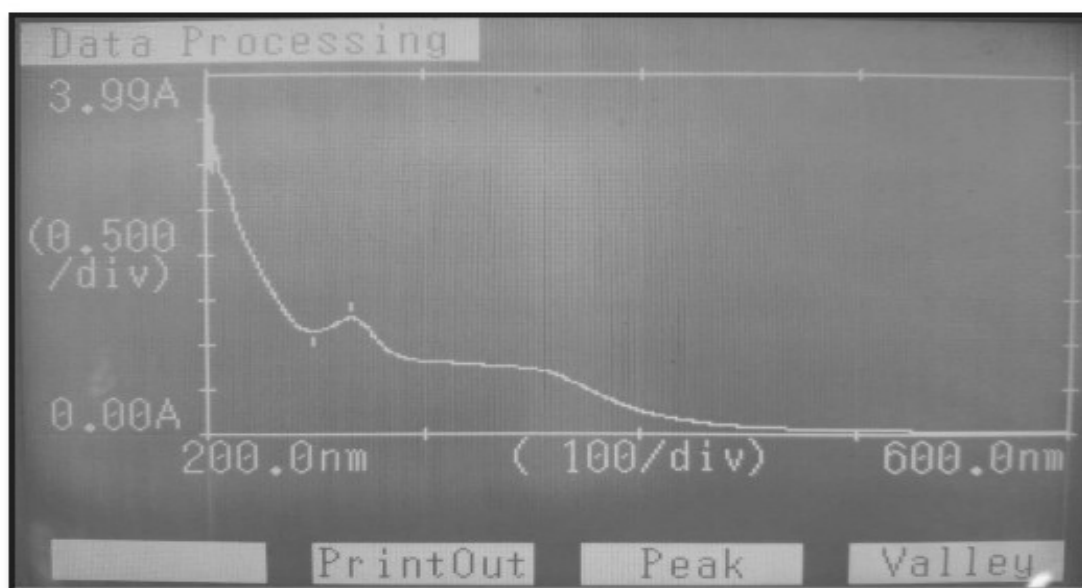
The prepared gel formulation was subjected to the percentage of drug content study. The results pointed out that all formulation do not show marked variation in their drug content. The results of antifungal activity are also given in the table. The results showed that with increase in plant extract concentration the zone of inhibition also increases and that value is comparable to the standard drug formulation.

#### Drug diffusion study:

Drug diffusion study was performed to determine drug diffusion across the egg membrane. The result showed that a very small amount of drug diffuses across the membrane i.e.  $2.8 \pm 0.1$  to  $0.87 \pm 0.02\%$ . The analysis of result revealed that low absorption is beneficial to avoid systemic effect. Hence, prepared gel formulation is found to be effective for the treatment of fungal skin diseases.

#### UV spectrophotometric evaluation:

The spectrum was taken in phosphate buffer solution pH 6.8. It showed distinct peak at 266 nm.

**Fig.5:-** UV spectra of C.tora.

**Development of calibration curve:**

It was developed in phosphate buffer solution 6.8 in UV range of 200-400 nm & absorbance was noted at 266 nm.

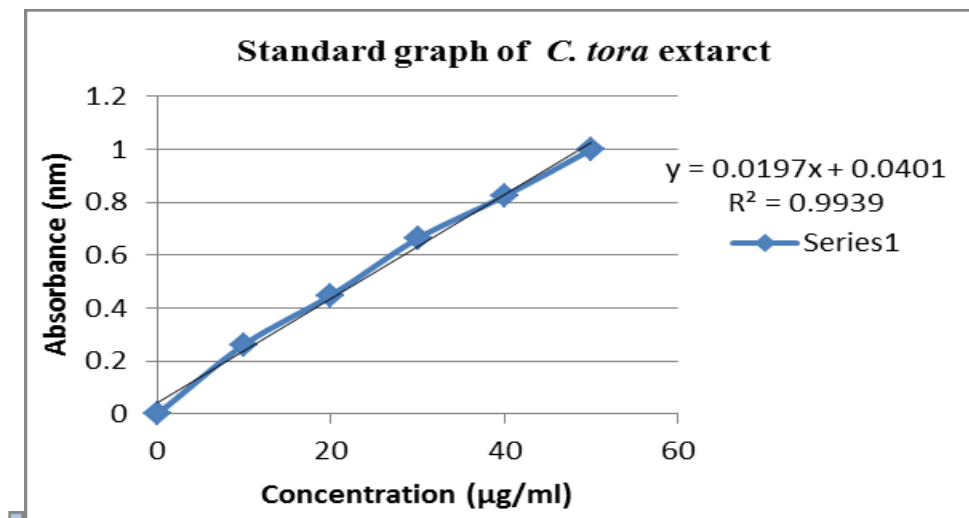


Fig 6:- Calibration curve of active constituent of *C.tora* at 266nm.

**In-vitro Antifungal activity:**

It was noted as discussed under the heading 'determination of zone of inhibition'.

Table 7:- Result of in-vitro Antifungal activity.

Formulation No.	Drug content %	Zone of Inhibition (mm) (Mean ± SEM)	
		Fluconazole (1mg/ml)	Plant extract (100mg/ml)
F1	95.20 ± 0.30	14.25 ± 0.50	12.23 ± 0.15
F2	94.76 ± 1.05	„	11.80 ± 0.65
F3	95.10 ± 0.85	„	12.05 ± 0.24
F4	95.55 ± 0.90	„	12.76 ± 0.45
F5	94.38 ± 0.60	„	11.38 ± 0.36
F6	97.40 ± 0.65	„	13.25 ± 0.50

The fungal strain tried against Methanolic extract of *C.tora* displayed zone of inhibition analogous to standard drug. Thus we can rationalize the fact that *c.tora* used age-old for fungal infection as allied by literature review.

**Short term stability studies:**

The study was conducted as per ICH guideline for the selected gel formulation F-6.

Table 8:- Result of Stability Studies.

S.No.	Formulation	Month	pH	Viscosity	Spreadability	Drug content
01	F6	0	6.45±0.15	680±45.50	38±2.0	97.40 ± 0.65
		1	6.45±0.17	680±42.35	37.5±0.10	97.22±0.34
		2	6.45±0.21	679±42.10	37.8±0.12	96.45±0.24
		3	6.45±0.18	678±41.08	37.3±0.13	96.23±0.15

During stability study, the pH, Spreadability and drug content was evaluated every month. The appearance was homogeneous and no significant changes were perceived in these parameters of optimized formulation (F6), indicating the stability of gel.

**Conclusion & Summary:-**

In present study, efforts were made to develop unique topical herbal gel formulation of *c.tora* extract for the treatment of common fungal skin disease. The observations can be summarized as below:

1. collected plant material was validated and found to be free from adulterant and substitute.
2. methanolic plant extract was made and tested for qualitative and quantitative analysis.
3. in vitro antifungal activity shown good result in selected fungal strain.
4. formulation of cassia tora topical gel was optimized and assessed for various parameters like pH, Spreadability, viscosity, homogeneity and drug content.
5. optimized formulation batch was exposed to short term stability studies as per ICH guideline and it displayed stability at different temperature and humidity.

Hence, formulation F6 having 300 mg carbopol 934 as gel forming agent and 4 ml propylene glycol as permeation enhancer may serve as operational topical gel formulation in common skin diseases.

The prepared gel formulation was found useful for topical application due to its decent Spreadability, homogeneity, neutral pH and low viscosity.

**Future prospective:**

Preclinical and clinical studies as well as the pharmacokinetic evaluation of the established gel formulation might be supported to optimize the desired effects related with topical gel formulation of cassia tora which may prove encouraging and worthwhile in future.

**Consent:**

Not applicable

**Ethical Approval:**

Not applicable

**Competing Interest:**

Authors have declared that no competing interests exist.

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