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FORMULATION AND CHARACTERIZATION OF ANTIDERMATOPHYTIC CREAM FROM ETHANOL EXTRACTS OF *CRESCENTIA CUJETE L.* DRIED LEAVES

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ABSTRACT

The use of natural products like plants as an alternative for prevention and cure of various diseases has been increasing over time. The present study aims to study phytochemical aspects of *Crescentia cujete L.* as the tree is known to have many active bio compounds that have enormous medicinal properties. The study emphasises on formulation of antidermatophytic cream using dried leaf extract of *Crescentia cujete L.* Antimicrobial and antioxidant property evaluations are carried out to prove the effects and applications of antidermatophytic cream formulated using ethanolic *Crescentia cujete L.* Leaves extract. The phytochemical analysis revealed the presence of alkaloids, flavanoids, carbohydrate, saponins, anthraquinones, glycosides, triterpenoids, tannins and phenolic compounds. The cream is envisaged to have stability and best capacity to reduce free radicals. The study demonstrates profoundly better antioxidant, antibacterial and antidermatophytic effects of ethanol extract of dried leaves of *Crescentia cujete L.* and formulated cream. Consistent activity was obtained in both ethanol extracts and formulated cream.

Keywords: *Crescentia cujete L.,* Antidermatophytic cream, Photochemistry, Antimicrobial, Antioxidant.

1. INTRODUCTION

Calabash tree or *Crescentia cujete L.* tree belongs to the family *Bignoniacea*. It is also known as the moonfruit tree. The calabash tree is 6 to 10 m tall with a wide crown and long branches covered with clusters of tripinnate leaves and gourd-like fruit. The branches have simple elliptical leaves clustered at the node. The greenish flowers arise from the main trunk and blooms at night [1]. Calabash fruit is a seasonal fruit that develops following pollination by bats. It appears at the end of dry season, and is 12 to 14 cm in diameter. It is globular with smooth hard green woody shell. It takes about six to seven months to ripen and eventually falls to the ground [1]. Small flat seeds are embedded in the pulp [2, 3]. The fruit is reported to have medicinal applications [2, 3]. Plant is propagated either by seed or stem cuttings.

Finding healing powers in plants is an ancient idea. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases [4]. According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants. Surprisingly, this large quantity of modern drugs comes from less than 15% of the plants which are known to have pharmaceutical value, out of the estimated 500,000 species of higher plants growing on the earth [5]. The free radicles of oxygen and nitrogen are naturally generated by human metabolism in various physiological conditions. They play an important role in the normal functioning of the body, such as in phagocytosis. When the production of these free radicals is exacerbated, the human body uses its efficient antioxidant system. However, in the oxidative stress there is an imbalance between the production of pro-oxidants and antioxidants, with predominance of pro-oxidants. This over production of free radicals can cause numerous problems to the body, such as aging and cell death, cancer induction, propagation of AIDS in HIV-positive patients and many others. The present study aims necessary to search for new sources of natural antioxidants, since many of the synthetic antioxidants in applicative dermal creams having different levels of anti-dermatophytic applications which are of concern.

2. MATERIAL AND METHODS

2.1. *Crescentia cujete L.* Leaves Extraction

Fresh and healthy leaves were collected from the plant found in the campus of Gujarat College, Ahmedabad.

The leaves of *Crescentia cujete L. w*ere dried using a drying hood. The dried *Crescentia cujete L.* leaves were mashed with a blender, and then *Crescentia cujete L.* leaf powder was obtained by filtering the power across a mess of muslin cloth in order to achieve fine powder. The ethanol extract was prepared by using 500 g of fresh leaves that were washed and stirred with 1000 mLof ethanol (analytical grade) in a blender for 15 min at room temperature and then filtered using analytical filter paper. Following this, the solvent (250 ml of ethanol) was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material was loaded into the thimble, which was placed inside the Soxhlet extractor. The side arm was lagged with glass wool. The solvent was heated using the isomantle and began to evaporate, moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reached the siphon it poured back into the flask and the cycle began again. The process should run for a total of 16 hours. Once the process had finished, the ethanol was evaporated using a rotary evaporator, leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask. The powder was percolated with 96% ethanol. After that, the obtained product was concentrated using a rotary evaporator to obtain a viscous ethanol extract of *Crescentia cujete L.* leaves. This paste was collected and stored in an ependroff tube or a sealed bottle at 10°C.

2.2. Preliminary Qualitative phytochemical screening[6-8]

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in leaf of *Crescentia cujete L.* The freshly prepared extracts were subjected to standard phytochemical analysis to ensure the presence of following phyto-constituents. Various phytochemicals were qualitatively estimated. Alkaloids were confirmed using Mayer's test, Wagner test and Dragendroff's test [8]. Carbohydrates estimation was achieved by Molisch's test, Fehlings test, Benedicts test and Barfoed test. Legal's test and Keller-Kilhani [8] test was performed to detect the presence of Glycosides. Based on review of literature, *Crescentia cujete L.*is found to have adequate amount of proteins and amino acids. These constitutents were estimated qualitatively and confirmed using Millon's test and biuret test [8]. Confirmation of flavanoids was done using Shinoda test and alkaline reagent test. [8] Libermaan-Burchard'test was performed to detect the presence of phytosterols and terpenoids. Polyphenolic compounds and tannins were confirmed using $FeCl_3$and lead acetate test [6]. Presence of anthraquinones and saponins was detected using NH_4OH and frothing, Emulsion test [7] respectively. Presence of active phytochemical constituent in leaf of *Crescentia cujete L.* was matched with previously reported studies [6-8].

2.3. Cream formulation

Base cream contains water and oil phases. The compositions and amounts of the formulation ingredients are shown in table 1. In order to prepare the cream, different amount of ingredients were incorporated together, and then the required amount of the herbal extract was added. 10g sample of the formulation was placed in a centrifuge tube (1cm diameter) and centrifuged at 2000 rpm for 5, 15, 30 and 60 min. Then the phase separation and solid sedimentation of the samples were inspected.

Table 1: Ingredients used for 10g antidermatophytic cream using *Crescentia cujete L*

Compound		Amount (g)
Oil phase	Stearic acid	1.0
	Glycerine monosterate	0.5
	Cetyl alcohol	0.5
	Liquid paraffin	0.5
	Bees wax	0.5
	Spermaceti	0.5
Water phase	Glycerin	0.5
	Methyl paraben	0.2
	Propyl paraben	0.2
	Water	5.5
	Rose oil	qs*
	*Crescentiacujete L.*extract	0.1

*Note: *qs= Quantity Sufficient*

2.4. Evaluation of cream

2.4.1. Thermal cycle test

The portion were stored at 5°C for 48 h and then at 25°C for 48 hour. The procedure was repeated 6 times and then their stability and appearance were evaluated.

2.4.2. Thermal change test

Three portions, each of 20 g, of cream formulation were stored at 4-6 °C, 25 °C and 45-50 °C respectively. Their stability and appearance were evaluated after 24 hours, one month and three months respectively.

2.4.3. Freezing and thawing

Known amountof cream formulation (20 g) was stored periodically at 45-50°C and 4°C for 48 hours. The procedure was repeated six times and then the samples were checked regarding their appearance and stability. Sample was subjected through a series of extreme, rapid temperature changes that it may encounter during normal shipping and handling processes.

2.4.4. Determination of pH

A suspension of each portion was prepared in 1% potassium nitrate solution to check pH value. A magnetic stirrer was used to produce homogeneity. Stability to pH was determined at 48 hours, one week and three week time duration.

2.4.5. Viscosity determination

Using a Brookfield viscometer (model DV-I with No. 6 spindle) the viscosity rheolog behavior of the cream was checked. Each sample was placed in a container and spindle velocity was raised gradually to maximum extent. Then the viscosity was determined at 0.3, 0.6, 3, 6 and 60 rpm. Student t-test (Microsoft excel software) was performed to compare test results with the control. $P<0.05$ was assumed as significant difference.

2.5. Antimicrobial Property

Staphylococcus aureus (ATCC No. 29737); *Bacillus cereus* (MTCC-430); *E. coli* (ATCC No. 8739); and *Salmonella typhi* (MTCC-733) were grown in Mueller Hinton broth (Merck, Germany) at 37°C for 24h. Final cell concentrations were 108cfu/ml according to the McFarland turbidometry. 100µl of the inoculum was added to each plate containing Mueller Hinton agar (Merck, Germany). Four different concentrations of the *Crescentia cujete L.* dried leaves extract and formulated cream (5, 10, 15 and 20% equal to 0.05, 0.1, 0.15 and 0.2mg/ml, respectively) were prepared. The sterile filter paper disks (6mm in diameter) were saturated with 50µl of each concentration of the extract and formulated cream. Water as solvent was used for preparing dilution at various concentrations. The plates were incubated at 37°C for 24h in an inverted condition and the diameters (mm) of inhibitory zones were measured. For standard, antibacterial agent, Streptomycin was used as broad spectrum antibiotic to carry out activity index of the extract and formulated cream. Antifungal or anti-dermatophyte properties were measured against test culture *Trichophytum rubrum* and *Microsporum canis* on Rose Bengal Agar using cup boarer method [9] (cup size 8mm). Inoculum of test culture was done using spread plate method. Standard broad spectrum antifungal agent Clotrimazole was used. Pure cultures of all test culture were procured from Department of Microbiology, Khyati Institute of Science, Ahmedabad, India. Activity index for antimicrobial assay was calculated using formula:

$$\text{Activity index [A.I.]} = \frac{\text{Mean of Zone of Inhibition by formulated cream suspension}}{\text{Zone of Inhibition obtained for standard Antibiotic Drug}}$$

2.6. Antioxidant Property

Antioxidant property of the formulated cream was evaluated using DPPH free radical scavenging assay, Hydrogen peroxide scavenging assay and Nitric oxide radical scavenging assay method.

2.6.1. DPPH free radical scavenging activity

The methodology described by Gulcin [10], was used with slight modifications in order to assess the DPPH free radical scavenging capacity of extract. Where bleaching rate of a stable free radical, DPPH was monitored at a characteristic wavelength in the presence of the cream. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH was prepared in ethanol and 0.5 mLof this solution was added 1.5 mL of formulated cream solution in ethanol at different concentrations (20-60 µg/mL). These solutions were vortexed thoroughly and incubated in dark. Half an hour later, the absorbance was measured at 517 nm against blank samples. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. A standard curve was prepared using different concentrations of DPPH. The DPPH concentration scavenging capacity was expressed as mM in the reaction medium and calculated from the calibration curve determined by linear regression (r2: 0.9845):

Absorbance = 9.692 × [DPPH] + 0.215.

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavanging effect (\%)} = \left[\frac{1 - As}{Ac}\right] x\ 100$$

Where "Ac" is the absorbance of the control which contains DPPH solution and "As" is the absorbance in the presence of extracts.

2.6.2. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out according to procedure by Ruch [11]. For this, a solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1

M, pH 7.4). Formulation cream suspension at the 20 µg/mL concentration in 3.4 mL phosphate buffer was added to 0.6 mL of H_2O_2 solution (0.6 mL, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing the sodium phosphate buffer without H_2O_2. The concentration of hydrogen peroxide (mM) in the assay medium was determined using a standard curve (r2: 0.9895):

Absorbance = $0.038 \times [H_2O_2] + 0.4397$

The percentage of H_2O_2 scavenging of extract and standard compounds was calculated using the following equation:

$$H_2O_2 \text{ Scavanging effect (\%)} = [\frac{1 - As}{Ac}] \, x \, 100$$

Where, "Ac" is the absorbance of the control and "As" is the absorbance in the presence of the sample or standards.

Hydrogen peroxide has strong oxidizing properties. It can be formed *in vivo* by many oxidizing enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. The ability of extract to scavenge hydrogen peroxide was determined according to the method of Ruch [10] as shown in Table 6 and compared with that of BHA, as standard.

2.6.3. Nitric Oxide Radical Scavenging Assay

The samples were prepared from a 0.1 mL formulated Cream in DMSO. These were then serially diluted with DMSO to make concentrations from 100-1000 µg/mL of each derivatives and the standard gallic acid. These were stored at 4°C for later use. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the drugs each derivative in DMSO (100-1000 µg/mL) and incubated at 25°C for 180 mins. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained drug at the same concentrations with no sodium nitroprusside. A volume of 150 µL of the reaction mixture was transferred and absorbance was measured at 546 nm using a UV-Vis spectrophotometer.

Gallic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging activity of each derivative of drugs and gallic acid were calculated using the following formula:

$$\text{Nictric oxide scavenged (\%)} = [\frac{Ac - As}{Ac}] \, x \, 100$$

Where, A control= absorbance of control sample and A standard= absorbance in the presence of the samples of drug derivatives or standards.

3. RESULT AND DISCUSSION

3.1. Preliminary phyto-constituent analysis

The phytochemical analysis of ethanol extracts of *Crescentia cujete L. d*ried leaves revealed the presence of alkaloids, flavanoids, carbohydrate, saponins, anthrax-quinones, glycosides, triterpenoids, tannins and phenolic compounds (Table 2). This result was in accordance with the previously reports [6-8].

Results of the phytochemical constituent determination of *Crescentia cujete L.* fruit is shown in Table 2. Phenol and tannin were present in the leaf sample. Phenol and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared in official test [12].

This may explain why *Crescentia cujete L.* was used as disinfectant and bactericides in emollient healing and in the treatment of burns [2, 3, 13]. Tannins have astringent properties that hasten the healing of wound, prevent decay and have antimicrobial activity [14].Tannins and their derivative compounds are known to be responsible for preventing and treating urinary tract infections and other bacterial infections [2, 13]. The presence of tannins in the leaves therefore suggests that it may serve as a useful antibacterial agent. Flavonoids found in *Crescentia cujete L.* can act as anti-oxidants and protect the cells of the body from free radical damage; free radicals are reputed to damage cell and contribute to various health related problems. The presence of alkaloid was observed in the leaf sample. Alkaloids are very important in medicine because, some alkaloids have been used as basic medicinal agent for their analgesic, anti-spasmodic and bactericidal effects [15]. Alkaloids in *Crescentia cujete L.* may explain why it is usedas analgesics in the treatment of coughs and as anti-inflammatory agents [2, 3, 13]. Saponins were present in *Crescentia cujete L.* Saponins are known to serve as natural antibiotics and also boost energy [16]. Saponins are also useful in reducing inflammation of the upper respiratory passage and also as foaming and emulsifying agent and detergents [15]. Saponins in *Crescentia cujete L.* may serve as anti-inflammatory agent and as antibiotics in

treating diseases and ailments. Anthraquinone were also present in the leaf sample. Anthraquinone on the other hand is used as laxative. The presence of anthraquinone in *Crescentia cujete L.* may explain why it is used as laxative [2, 3, 13].

Table 2: Phytochemical analysis of ethanol extracts of *Crescentia cujete L.* dried leaves

Phytochemical	Test	Observation	Presence
Alkaloids	Mayer's test	White creamy precipitate	+
	Wagner test	Reddish brown precipitate	+
	Dragendroff's test	Yellow/orange precipitate	+
Carbohydrates	Molisch's test	Violet ring	+
	Fehling's test	Red precipitate	+
	Benedicts test	Red precipitate	+
	Barfoed's test	Red precipitate	+
Glycosides	Legal's test	Blue coloration	+
	Keller-Kilhani test	Reddish brown ring	+
Protein and Amino acids	Millon's test	White precipitate	+
	Biuret Test	Pink colour in ethanol layer	+
Flavanoids Test	Shinoda test	Pink scarlet, crimson red colour appears	+
	Alkaline reagent test	Yellow colour to colourless with dil.HCl	+
Phytosterols	Libermaan-Burchard'test	Brown ring with upper green layer	+
Terpenoids	Libermaan-Burchard'test	Brown ring with deep red layer	+
	Salkowski test	Yellow coloured lower layer	+
Phenolic Compounds and tannins	$FeCl_3$test	dark green colour	+
	Lead acetate test	White precipitate	+
Saponins	Frothing test	Persistent froth	+
	Emulsion test	Emulsion layer	+
Anthraquinones	NH_4OH test	Pink, red or violet colour in the ammoniac (lower) phase	+

3.2. Formulation and physicochemical evaluation

The main problems with the preliminary base formula were the excess fat content which produced a greasy sense on usage, turbidity and its low consistency. Therefore, the formula was modified to overcome the problems. At first, the proportions of the oil phase components were changed. Liquid paraffin was also added to produce better flow properties.

The results showed that by decreasing the amount of liquid paraffin, the stiffness and consistency of the formula were increased. The best concentration was chosen to be 1.5 gm of liquid paraffin. However addition of bees wax made it more precisely viscous with preferable consistency. 0.5 g Glycerine mono-sterate was added to enhance the stability of the formula. Consistent pH is found as 6.8 with base as well as formulation (F_1) for the checked duration of 24 hours, 1 week and 3 weeks respectively. Thermal change test shows stability in the cream as performed for the exposure of various temperatures at 4-6°C, 25°C and 45- 50 °C of storage for 24 hours, 1 week and 3 weeks duration respectively. More over there is also a stability found for thermal cycle test where cream was stored at 5°C for 48 h and then at 25°C for 48 h and then their stability and appearance were observed stable even though the procedure was repeated 6 times.

Results of viscosity determination as measured at various rpm of 0.3, 0.6, 3, 6, 60; showed considerable decrease in viscosity (cps) with reference to duration. The results for determination of viscosity are given in table 4.

The study reveals that, natural source for excipient is more suitable due to easy availability, non-toxicity and biocompatible properties of excipient. After studying the physical parameters like stability, it was concluded that the product has the best capacity to reduce free radicals and can be used as the provision of a barrier to protect skin.

3.3. Antioxidant Property of formulated cream

Antioxidants are closely related to their bio functionalities, such as the reduction of chronic diseases like DNA damage, mutagenesis, carcinogenesis and inhibition of pathogenic bacteria growth which is often associated with the termination of free radical propagation in biological systems [17].

3.3.1. DPPH free radical scavenging activity

The antioxidant activity of ethanolic extracts of fresh leaf and formulated antidermatophytic cream from *Crescentia cujete L.* were assessed using DPPH radical scavenging activity. The results were shown in the Table 5. Ethanol extract of fresh leaves of *Crescentia cujete L.* and formulated antidermatophytic cream were found to possess antioxidant property in biological systems. It can scavenge free radicals and has the ability to sequester redox active metal Ions [18-19]. Free radical scavenging activity was evaluated by performing *in-vitro* DPPH assay. The IC_{50} value of the formulated cream is found to be much lesser *i.e.* 256µg/ml and that of ethanolic extract is 400µg/ml which is even found more than the standard ascorbic acid used.

Table 3: Physicochemical and stability evaluation of Cream (n=5)

Property	Formulation	
	Base	F_1
Homogeneity test	Hetrogenous	Homogenous
Creaming and Coalescence	Stable	Stable
Centrifugation Test	Stable	Stable
Thermal cycle Test	Separated	Stable
Freezing and Thawing	Stable	Stable
pH (average)	6.8 (consistent)	6.8 (consistent)
Long term Stability	Not determined	Stable
Fluidity	Fluid	Medium viscous
Appearance	Soft and fatty	Clear good flow

Table 4: Periodically viscosity monitoring of the final formulation (mean ± SD, n=3)

Shear rate (rpm)	Viscosity (cps)			
	After 2 days	After 1 week	After 1 month	After 3 months
0.3	521.00 ± 0.05	503 ± 0.24	537 ± 0.13	549 ± 0.07
0.6	211 ± 0.01	184 ± 1.19	192 ± 0.58	226 ± 0.05
3	9.51 ± 0.25	11.32 ± 0.01	15.65 ± 1.09	21.07 ± 0.02
6	2.54 ± 0.04	2.19 ± 0.5	2.66 ± 0.02	2.43 ± 0.01
60	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.001	0.6 ± 0.2

Table 5: DPPH free radical scavenging activity of ethanol extract of fresh leaves of *Crescentia cujete L.* and formulated antidermatophytic cream

Sample	Concentration (µg/ml)					IC_{50} VALUE
	0	250	500	750	1000	
Ethanol Extract	31.06	43.91	54.39	67.09	83.16	400
Formulated cream	34.21	49.87	61.83	73.19	81.06	256
Control (Ascorbic Acid)	27.42	46.48	63.64	76.22	84.85	300

3.3.2. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging assay results showed that extract had an effective hydrogen peroxide scavenging activity. At the above concentration, the hydrogen peroxide scavenging effect of extract and four standard compounds decreased in the order of control > extract > formulated cream. Hydrogen peroxide itself is not very reactive; however it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells.

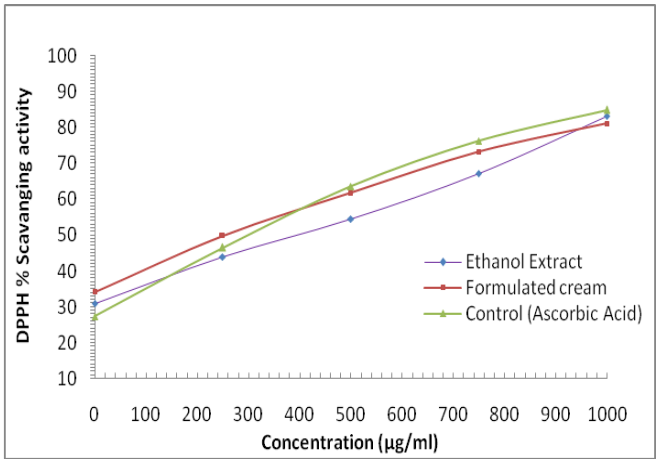
3.3.3. Nitric Oxide Radical Scavenging Assay

Nitric oxide (NO), being a potent pleiotropic mediator in physiological processes and a diffusible free radical in the pathological conditions, reacts with superoxide anion and form a potentially cytotoxic molecule, the 'peroxynitrite ($ONOO^-$). Its

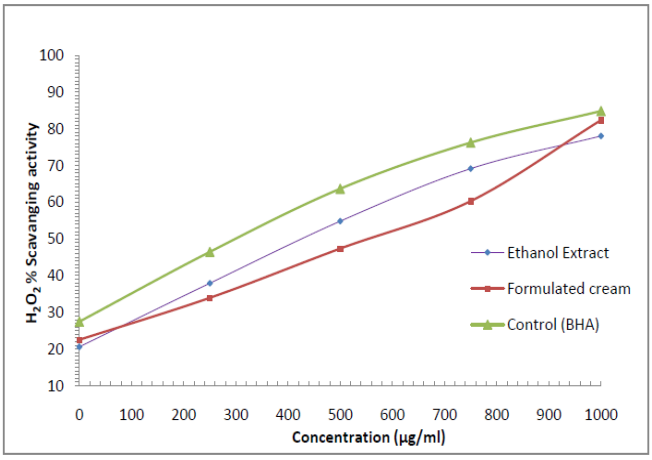
protonated form, peroxynitrous acid (ONOOH), is a very strong oxidant [20]. The main route of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. Under physiological conditions, peroxynitrite also forms an adduct with carbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins [21-22] in living systems. Since the reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity, the reducing ability of phytoextracts is measured in this study. The antioxidant activity has been reported to be concomitant with development of reducing power. The antioxidant activity in terms of nitric oxide scavenging assay, formulated cream showed more activity than extract. IC_{50} value of the formulated cream is lessas compared to ethanol extracts of *Crescentia cujete L.* fresh leaves.

Table 6: Hydrogen peroxide scavenging activity of ethanol extract of fresh leaves of *Crescentia cujete L.* and formulated antidermatophytic cream

Sample	Concentration (µg/ml)					IC_{50} VALUE
	0	250	500	750	1000	
Ethanol Extract	20.56	37.91	54.82	69.16	78.05	428
Formulated cream	22.51	33.94	47.34	60.27	82.36	555
Control (BHA)	27.42	46.48	63.64	76.22	84.85	295



Graph 1: *In-vitro* antioxidant activity by DPPH method



Graph 2: *In-vitro* antioxidant activity by H_2O_2 method

Table 7: Nitrogen Oxide Radical scavenging activity of ethanol extract of fresh leaves of *Crescentia cujete L.* and formulated antidermatophytic cream

Sample	Concentration (µg/ml)					IC_{50} VALUE
	0	250	500	750	1000	
Ethanol Extract	26.19	43.89	59.72	72.16	86.31	344
Formulated cream	30.02	46.18	59.87	72.06	83.45	316
Control (Gallic Acid)	24.37	48.84	69.1	79.42	89.12	260

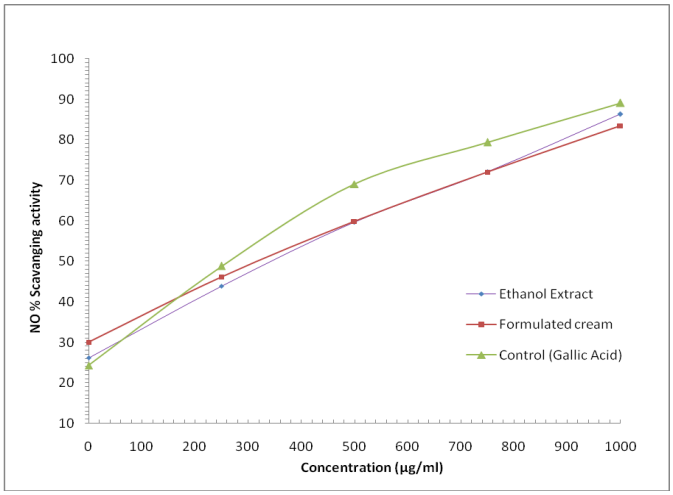
Based on the above results, antioxidant capacity is widely used as a parameter for medicinal bioactive components. Antioxidant neutralizes free radicals, unstable oxygen molecules that break down skin cells and cause wrinkles, thus preventing impairment at the cellular level. In the present work, face cream formulations were prepared using antioxidant from natural source using *Crescentia cujete L.* fresh leaf ethanol extract. These *in-vitro* assays demonstrate that plant extracts are important sources of natural antioxidants, which might be useful as preventive agents against oxidative stress and hence currently the evaluation of *in-vivo* antioxidant activity of these extracts is in progress. To elucidate the prime source of antioxidant

properties further studies should be carried out with isolate active principles.

3.4. Anti-dermatophytic property

The healing power of plants is a widely explored. Plants have been traditionally used for the treatment of infection of different aetiology. So with the development of bacterial resistance of some microorganisms is mainly to the abuse of antibiotic use. The increasing prevalence of multidrug-resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotic raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies as cited by Mahbub [23].

Activity index of ethanol extract of fresh leaves of *Crescentia cujete L.* and formulated antidermatophytic cream were checked against test cultures with reference to standard antibacterial and antifungal streptomycin and Clotrimazole respectively. The test cultures were selected to check antibacterial and antidermatophytic properties. Consistent activity was obtained in both ethanol extracts and formulated cream. However, higher concentration greater than 10% *i.e.* 0.1 mg/ml concentrations showed greater influence as compared to standards.



Graph 3: *In-vitro* antioxidant activity by NO method

Table 8: Antimicrobial property of extract and cream from *Crescentia cujete L*

Test Culture	Diameter of Zone of inhibition (mm) [(Mean ±SD; n=3]							
	Ethanol extract – *Crescentia cujete L.* dried leaves				Formulated Cream (F_1)			
	5 %	10 %	15 %	20 %	5 %	10 %	15 %	20 %
S.aureus (ATCC No.29737)	8.36±0.02 [*0.684]	9.02±1.03 [*0.738]	12.84±0.08 [*1.050]	13.46±0.02 [*1.101]	8.22±0.42 [*0.672]	8.92±0.01 [*0.729]	13.97±0.12 [*1.143]	13.03±1.03 [*1.066]
Bacillus cereus (MTCC-430)	6.19±1.07 [*0.506]	8.13±0.04 [*0.665]	10.08±0.21 [*0.824]	10.46±0.07 [*0.855]	6.49±0.23 [*0.531]	9.24±0.05 [*0.756]	11.33±0.01 [*0.927]	12.07±0.06 [*0.987]
E. coli (ATCC No. 8739)	7.06±0.01 [*0.577]	8.22±0.03 [*0.672]	9.11±0.05 [*0.745]	12.03±1.05 [*0.984]	8.61±0.08 [*0.704]	10.7±0.11 [*0.878]	12.54±0.18 [*1.026]	14.93±0.01 [*1.221]
Salmonella typhi (MTCC-733)	6.04±0.07 [*0.494]	8.14±0.05 [*0.666]	8.67±0.02 [*0.709]	9.71±0.08 [*0.794]	6.04±0.01 [*0.494]	8.62±0.02 [*0.705]	8.20±0.05 [*0.671]	8.91±0.01 [*0.729]
Trichophytum rubrum	8.20±0.01 [*0.622]	9.18±0.20 [*0.696]	11.56±0.07 [*0.877]	13.51±0.01 [*1.025]	8.91±0.05 [*0.676]	10.3±0.20 [*0.779]	12.64±0.01 [*0.959]	13.83±0.07 [*1.049]
Microsporum canis	9.07±0.01 [*0.688]	11.73±0.05 [*0.889]	13±0.07 [*0.986]	13.74±0.01 [*1.042]	8.90±0.08 [*0.675]	12.61±0.02 [*0.956]	13.94±0.01 [*1.057]	14.37±0.02 [*1.090]

**Zone of Inhibition by Streptomycin as a standard drug = 12.22 mm (Mean Value), - Zone of Inhibition by Clotrimazole as a standard drug = 13.18 mm (Mean Value), - [*] indicates Activity index (A.I.)*

4. CONCLUSION

The study demonstrates profound antioxidant, antibacterial and antidermatophytic effects of ethanol extract of dried leaves of *Crescentia cujete L.* and formulated cream. Comparatively there is more activity index in formulated cream rather than ethanol extracts with reference to standard broad spectrum controls. Moreover, based upon IC_{50} values and antioxidant assay results the cream is more profoundly preferable as compared to ethanol extract. Natural source for excipients is more suitable due to easy availability, non-toxicity and biocompatible properties of excipients. After studying the physical parameters like stability, it was concluded that the product has the best capacity to reduce free radicals and can be used as the provision of a barrier to protect skin as profound and significant

antimicrobial properties were achieved. Findings of the present study also provided the scientific evidence that natural plants could be used in traditional medicine for the prevention and treatment of dermatophytic infections. However, further research on long term stabilities of physical, chemical and antifungal activities of the *Crescentia cujete L.* antidermatophytic cream should be conducted to find the optimum storage condition. The clinical trial of the cream could also be tested on the affected patients to explore the practicality of the cream and the possibility of commercial development.

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Conflicts Of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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