Development and Evaluation of Aloe-Vera Gel Loaded Crack Cream

DURGESH W. MOHARKAR¹, ASHISH D. LANDE², PRANALI D. SHAHARE³, DR. MOHAMMAD TAUQEER SHEIKH⁴, ADESH S. MESHRAM⁵

1, 2, 3, 4, 5 Dr. Arun Motghare College of Pharamcy Kondha-Kosara

Abstract- Objective: The main objective of the work was "ToPrepare and Evaluate Aloevera Crack cream".

Method: The dried juice or latex collected by incision from the bases of the leaves of Aloe barbadensis Miller were collected and the the plant was authenticated, collected leaves were washed, shade dried and used for the further investigation. The dried juice sample was grinded using mechanical grinder to skimmed liquid. The extract containing different concentration of Aloe barbadensis Miller was investigated against Staphylococcus aureus for Antibacterial activity. The leaf extract of Aloe barbadensisMiller showed the antibacterial activity against bacteria such as Staphylococcus aureus.

Conclusion: We conclude that extract of Aloe barbadensis Miller is effective against bacteria and have the potential in the treatment of bacterial and fungal types of Skin diseases, Agriculture and Food industry.

Indexed Terms- Aloevera, Polyherbal cream, cracked heel, Ghee & Cream Formulation.

I. INTRODUCTION

• TRANSDERMAL SYSTEM

The skin is the body's first line of defense for external exposure. The signs of ageing are most viable in the skin. Although ageing skin is not a threat to a person, but it can have detrimental effect on the psychology of a person.^[1]

Also, feet is an important organ of the human body and are exposed to lots of friction and external environment. The lacks of oil glands on the sole of foot predispose it to the dry skin. Negligence to the feet can lead to different disorders generally due to improper footwear, and one can suffer from infections because of an external penetration of dirt, fungus, bacteria through these cuts and wounds. It is reported that the bacterial decomposition give rise to foot odor, in which bacteria Staphylococcus Epidermis is responsible. Also, foot resident microorganisms are responsible for infections such as Candida albicans, Escherichia coli and Staphylococcus aureus.^{[2],[3].}





Fig 1. Healthy VS Unhealthy Skin conditions.

• Crack heels:

Cracked heels, also referred to as heel. Fissures are a common foot condition, which con Cause discomfort on even pain. They result from dry skin & are accompanied by thicked skin, plus sometimes yellow on brown calluses around the heel edge. Often, the only problem with cracked heels is their appearance. Cracked heels can occur for a number of reasons from lacking enough moisture to exposing footwear. If you have noticed symptoms such as dry skin thickening around heels, or have cracked skin or heel pain, then the causes can help you understand the condition. It can also prevent further relapses from happening.

• Most common causes of cracked heels:

There horse several potential too moist you may get a bacterial o fungal infection. This can cause cracks between the toes. People who often walk bare foot or wear open shoes are of risk for dry skin. people who wear shoes without socks on shoes Water on your feet can Take away natural oil from the skin & can leave the skin rough or dry,

- Poor fitting of shoes: Wearing shoes that do not fit properly can contribute to cracked feet.
- Genetics: Naturally dry or thick, dry skin (Callus) around the heels could be genetic cause of cracked heels.^[4]
- What are Cracks?

Cracks are breaks in your skin. They may be the result of skin that is too dry. When skin is too dry it can become rough and flakey. A large fissures often forms on the base of the heel. Take a look at your routine & if you identify anything affecting you then try altering your current habit.

- Lacking moisture: Most common cause of cracked heels, The skin underneath your feet is often dry, rough, or chopped. This is because the skin around your heels has a relatively small number of sweat glands, If skin is particularly dry then cracked skin are appear more easily options more easily to being less elasticity and can be exacerbated by any of the below problems.
- Deficiencies: Lack of vitamins, minerals & zinc in your diet can adversely affect your heel health.
- Pressure: Spending a long time standing at work or home con stress the skin on heels.
- Ageing skin: Thick, dry, Scaly Skin loses its elasticity with age and such crack can have higher incidence as you become older.
- Disorders: Athlete's foot psoriasis eczema thyroid disease diabetes and some other skin condition can be the cause of cracked heels.
- Obesity: Being overweight can increase pressure on the fat pad under the heel. This can cause it to expand sideways and increases of the skin lacks Flexibility put pressure on the feet.
- Hygiene: Failing to keep your feet adequately clean can be further cause of cracked heels. ^[5]

ALOE VERA

Aloe vera, locally known as Guarpatha, is a cactus like plant that grows in a hot, dry climate. As a result of its use as a folk medicine, it is claimed that Aloe vera has wound and burn healing properties, antiinflammatory and immuno-modulatory effects.The aloe vera plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. It is a natural product that is nowadays frequently used in the field of cosmetology. It can be applied topically as an emollient for burns, sunburn and mild abrasion and for inflammatory skin disorder. It has antifungal, antiviral, antioxidant and anti-inflammatory effects. Aloevera is used externally for its wound healing properties and is supported by clinical investigation. The plant is the source of two commonly used products that differ in their chemical composition as well as therapeutic abilities - these two products are the gel and yellow sap. The gel or juice is clear viscous material that is separated from the parenchymal tissues in the leaves of plant.

The second product, yellow sap or latex, present in the pericyclic tubule cell, is an active cathartic pharmaceutical product known simply as the Aloe.

It contains more than 75 potentially active constituents mainly present in the gel and in the yellow sap of the plant. Polysaccharide in the gel helps in binding moisture into the skin. Aloe stimulates fibroblasts which produce collagen and elastin fibre making the skin more elastic and less wrinkled. It also has cohesive effect on the superficial flaking epidermal cells by sticking them together, which softens the cells by sticking them together, which soften the skin. The amino acid also softens harden skin cell and the zinc act as astringent to tighten the pores.

In these studies, attempts have been made to develop foot care crack cream from the herbal extract. Plants and their extracts have immense potential for the management and treatment of skin disorders. The phyto-medicines for wound healing are not only cheap and affordable but are also safe as hypersensitive reactions are rarely encountered, with the use of these agents WHO has been promoting traditional medicines as a source of less expensive, comprehensive medical care, especially in developing countries. Eight percent of the world population relies on medicinal plants for their primary healthcare. WHO also recognized the importance of traditional medicine and has treated. ^[6]

PLANT PROFILE :



Fig 2. (a) Incised plant showing late, (b) aloevera plant

Taxonomic Classification

- Kingdom:Plantae
- Clades:Tracheophytes
- Clade: Angiosperms
- Clade :Moncots
- Order :*Asparagales*
- Family: *Asphodelaceae*
- Subfamily: Asphodelaceae
- Genus:Aloe
- Species:A.vera
- Scientific name: *Aloe barbadensis Miller, Aloe perryi Baker, Aloe ferox Miller.*
- Common name: *Aloe Musabbar, kumara*.^[7]

DESCRIPTION:

Aloevera is a steamless or very short-steamed plant growing to 60-100 (24-39m) tall, spreadingby offsets.^[8] The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on their upper & lower stem surfaces.^[9] The margin of leaf is serrated and has small white teeth. The flower are in summer on a spike up to tall 90 cm. (35in)tall each flower being pendulous, with a yellow tubular corolla 2-3 cm (0.8-1.2 in) long.^[9 & 10] Like other Aloe Species. Aloevera forms arbuscularmycorrhiza, a symbiosis that allows the plant better access to mineral nutrient in soil. [11]

• PREPERATION AND EVALUATION OF ALOE VERA CRACK CREAM:

Aloevera is known for its skin care and medical properties since time immemorial the plant is extensively recognized as a remedy for burns rashes allergic irritation Skin aging, wounds infection sunburn, eczema, psoriasis, dermatitis &inflammation. It is also used as good moisturizer for dry skin for lightening blemishes and scars as well as hair growth promoter.

II. MATERIALS AND METHOD

MATERIALS:

- Fresh leaves of Aloevera were procured from Botanical Garden of MIBP, Gondia.
- All the other ingredients like cetyl alcohol, stearic acid, glycerine, KOH, triethanolamine, were supplied from the Laboratory of Pharmaceutics, MIBP, Gondia
- All the chemicals used in study were of analytical grade.

PREPERATION OF ALOEVERA EXTRACT:

- Aloevera leaves were cut, washed and then sliced an inch both on upper and lower sides.
- The leaves were further cut and the pulp was removed thereafter.
- The obtained pulp was further crushed in mixer grinder.
- After crushing, it was strained in order to remove the attached fibers.
- The aloe juice thus prepared was heated at 50-60°c and is stirred continuously.
- Then the obtained juice was stored in refrigerator for further use.

CREAM FORMULATION:

The formulation of the cream contains the following contents:

- Aloe Juice: It is used for its antibacterial, antioxidant and wound healing properties.
- Glycerine: It is used in the formulation as humectants as well as smoothening agent.
- KOH (Potassium Hydroxide): It provides emulsifying property to the formulation.
- Stearic acid: It is used as a cream base in the formulation.

- Cetyl alcohol: It has different uses like skin softening, soothing, and healing of the dry skin.
- Triethanolamine: It is used for the pH adjustment of the formulation.
- Borax: It is used as Emulsifier, buffer and preservative.
- Ghee: It is used for the moisturisation, hydration, cure and slowing down of ageing of the skin.
- Rose water: It is used as perfuming agent in the formulation.

PROCEDURE :

- Oil in water (o/w) emulsion based cream (semisolid) formulation was formulated.
- The emulsifier (stearic acid) and other oil soluble components (cetyl alcohol, ghee) were dissolved in oil phase (Part A) and heated at 75°c.
- The preservatives and other water soluble components (borax, triethanolamine, glycerine, KOH, aloe extract) were dissolved in aqueous phase (Part B) and heated to 75°c.
- After heating, the aqueous phase was added in portions to oil phase with continous stirring until cooling of emulsifier takes place.

INGREDIENTS	F1	F2	F3	F4	F5
Aloe extract(ml)	35	36	37	36	34
Glycerine(ml)	4	4.5	4.6	4.5	4.5
KOH(g)	0.18	0.2	0.3	0.2	0.4
Stearic acid(g)	8.0	8.5	8.0	8.5	8.0
Cetyl alcohol(g)	0.25	0.3	0.4	0.3	0.5
Ghee(g)	1.5	2.0	2.5	2.0	2.5
Triethanolamine(ml)	0.6	0.7	0.8	0.7	0.9
Borax(g)	0.04	0.08	0.085	0.08	0.16
Rose water(ml)	0.1	0.1	0.1	0.1	0.1

Table 3. The formula for the preparation of cream is given below:

EVALUATION OF CREAM

PHYSICO-CHEMICAL EVALUATION PARAMETERS:

- 1. Appearance: The appearance of cream was judged by its color, pearlescence and roughness.
- 2. pH: The pH meter was calibrated using standard buffer solution. About 0.5g of cream was weighed and dissolved in 50 ml of water and its pH as measured.
- 3. Spreadability: Spreadability of formulations was determined with a modified apparatus consisting of wooden block provided with two glass slides. Lower slide was fixed on wooden block and upper slide with one end tied to weight pan. A cream equivalent to 2.5g was placed between two slides and 1000g weight was placed over it for 5 minutes to press the sample for its uniform thickness. Weight of 80g was added to pan. The time (in sec) require to separate the two slides was taken as a measure of spreadability. Shorter

time interval to cover the distance of 7.5cm indicates better spreadability.

- The spreadability (S) can be calculated using the formula,
- $\mathbf{S} = \mathbf{m} \mathbf{x} \mathbf{L} / \mathbf{T}$
- Where, S-spreadability, m-weight tied to upper glass slide, L- length moved on glass slide, T- time taken.
- The determinations were carried out in triplicate and the average of three readings were recorded.
- 4. Washability: This test is carried out by simply washing applied cream with water.
- 5. Determination of type of smear: It was determined by applying the cream on the skin surface of human volunteers. After application of the cream, the type of film or smear formed on the skin were checked.
- 6. Homogeneity: The formulations were tested for the homogeneity by visual appearance and touch. [12]

- 7. Viscosity: Viscosity of the formulation was determined by Brookfield viscometer at 100rpm and by using spindle no.7.The sample (50g) was placed in a beaker and was allowed to equibrase for 5 minutes. Before measuring the dial reading using a T-D spindle (no.7) at 100rpm at speed, corresponding dial reading on the viscometer was noted. The measurements were carried in temperature. triplicates at room Direct multiplication of the dial readings with factors is given in the Brookfield viscometer. Catalogue gave the viscosity in centipoises.^[13]
- 8. Irritancy test: Mark an area (one sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema was checked if any for regular intervals upto 24 hours and reported.

DETERMINATION OF THE TYPE OF EMULSION

- 1. Dye solubility test: In this test, an emulsion is mixed with water soluble dye (amaranth) and observed under the microscope. If the continous phase appears red, it means that emulsion is oil in water (o/w) type as the water is in the external phase and the dye will dissolve in it to give colour. ^{[1],[13]}
- 2. Accelerated stability test: Accelerated stability testing of prepared formulation was conducted for 2 most stable formulations at room temperature, studied for 7 days. They were formulation 4 and 5 at 40°c for 20 days. The formulations were kept both at room temperature and elevated temperature and observed on 0th, 5th, 10th, 15th, and 20th day for the following parameters:^[15]

OBSERVATIONS:

Table	4.	Observ	ation	of	evaluation	F	parameters:
	PARAMETERS	F1	F2	F3	F4	F5	7
	Appearance	Good	Good	Good	Great	Great	_
	pH	5.47	5.75	5.92	5.78	5.54	1
	Spreadability	Fair	Good	Good	Good	Good	7
	Washability	Washable	Washable	Washable	Washable	Washable	7
	Type of smear	-	-	-	-	-	7
	Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenou	s
	Emulsion type	o/w	o/w	o/w	o/w	o/w	7
	<u></u>	•	-	The entimie	chiel estivity	f frach gal a	falcovero

ANTIMICROBIAL EVALUATION:



Fig 3. Antimicrobial activity. (Staphylococcus aureus)

The antimicrobial activity of fresh gel of aloevera and the creams formulated with these extract were evaluated. The preliminary in vitro antimicrobial activity of the extract at various concentration and those of their creams were determined against some microorganisms using the agar cup plate method. The minimum inhibitory concentration (MIC) was also determined by agar dilution method. The physical properties of creams formulated with these extract was evaluated using standard procedures.

Procedure: For screening, the dilution of the extract was prepared. For this 0.2g of cream was weighed and to this 0.8ml of sterile distilled water was added. Antimicrobial activity of cream was observed against various microorganisms specially S.aureus by cup plate method, for studying zone of inhibition.

© DEC 2022 | IRE Journals | Volume 6 Issue 6 | ISSN: 2456-8880

Microorganisms were grown in a suitable agar medium. The wells were filled with the diluted formulation. The plates were incubated at 37°c for 48 hours. The activity of cream is indicated by a clear zone of inhibition around wells, this zone of inhibition was recorded.^[16]

Table 5	T-1-1-	£	A	
Table 5.	I able	IOr	Antimicrobial	evaluation:

S	Test	Positiv	Plant	Zone of inhibition in				
r	Cultu	e	extra	(mm)				
	re	control	ct 0.1	DM Posi		Plant		
Ν		0.1 ml	ml	SO	tive	extract		
0		for	for					

		(1	(1mg	Sol	Con	
		mg/ml)	/ml)	ven	trol	
				t	(cm	
)	
1	<i>S</i> .	Strepto	Aloe	-	2.4	1.8
	aureu	mycin	Extra			
	S		ct			

The Aloe extract showed mild to moderate antibacterial activity against *Staphylococcus aureus* with 2.4 cm.

Table 6:	Minimum	Inhibitory	Concentration	of A.	barbadensis Miller.
----------	---------	------------	---------------	-------	---------------------

Sr.no.	Microorganism	Con	Concentration(µg/ml)						MIC(µg/ml)			
		50	100	150	200	250	300	350	400	450	500	
1.		+	-	-	-	-	-	-	-	-	-	100
	S.aureus											

Turbidity: Present = (+) Absent = (-) Formulation and evaluation of cream containing extract for antibacterial activity. Characterization of Cream:

Table no. 7: Colour and Appearance :-

Test	Result
Colour	Creamy White
Appearance	Semisolid cream

Table No. 8: Homogeneity :-

All developed cream were tested for homogeneity by visual inspection after the cream is have been set in the container.

Sr.No.	Batch	Ph
1	F4	Homogeneous
2	F5	Homogeneous

Table No. 9: pH of the formulation :-

Subj	ject			Parameter fo	arameter for the evaluation of product							
Sr. no	Se x	Ag e	Ski n type	Appearanc e	Spreadabilit y	Skin change s	Irritanc y	Healing property	Moist proper	urizing ty	Drynes s of soles	
1.	М	47		Dry	Good	Good	Appear s	-	Grea t	Observe d	-	
2.	М	32		Healthy	Good	Good	Appear s	-	Goo d	Observe d	-	
3.	F	40		Healthy	Good	Good	Appear s	-	Goo d	Observe d	-	

The pH of cream was determined by digital pH meter/gm of cream was dissolved in 50 ml distilled water and the pH was measured.

© DEC 2022 | IRE Journals | Volume 6 Issue 6 | ISSN: 2456-8880

Sr. No.	Batch	pН
1	F4	5.78
2	F5	5.54

Table No. 10: Spreadability of formulation :-

Sr.No.	Batch	Spreadibility
1	F4	Easily spread
2	F5	Easily spread

Table No. 11: Viscosity :-

Sr. No.	Batch	R P M	СР
1.	F4	10	3953
2.	F5	20	3957

SUBJECTIVE EVALUATION:

From antimicrobial observation the formulation with highest ability was selected for evaluation. Foot cream was given to subjects to use to carry out the evaluation. These subjects were asked to use the cream for a week and note down the changes before and after the use of aloevera crack cream. They were asked to evaluate the product on the basis of parameters like appearance of the product, spreadability of cream, skin changes on feet and irritancy. They were asked to rate the product on its overall performance, the data was interpreted as 5 points for excellent, 4 for very good, 3 for good, 2 for fair and 1 for bad performance.^[17]

Table 12.	Subjective eveluet	tion with rannat to	different peremeters
	Subjective evaluat	non with respect to	different parameters.

Subject Parameter for the evaluation of product					et					
Sr. no	Se x	Ag e	Skin type	Appearanc e	Spreadabilit y	Skin changes	Irritanc y	Healing propert y	Moisturizin g property	Drynes s of soles
1.	М	47	Dry	Good	Good	Appear s	-	Great	Observed	-
2.	М	32	Health y	Good	Good	Appear s	-	Good	Observed	-
3.	F	40	Health y	Good	Good	Appear s	-	Good	Observed	-

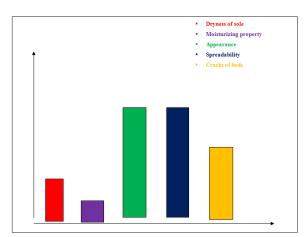


Fig 4 :Statistical representation for subjective evaluation of aloevera crack cream :

After one week, information regarding allergy or any other unwanted condition were collected from the subjects. All the adverse events reported or observed by subjects were recorded with information about severity, date of onset, duration and action taken regarding the study of cosmetic. The results were compiled from Table. 1.

Result

- pH of cream: The pH of cream was found in range of 5.6 to 6.8 which is good for skin pH. All the formulation of cream were shown pH nearer to skin required i.e. pH of F1-, F2-, F3-, F4 and F5-.
- Appearance: The cream prepare was found to be creamy white in color and has pleasant odor.
- Viscosity: the viscosity of the cream in range of 28001-27025 cps which indicate that the cream is easily spreadable by small amount of shear. But F4 & F5 shows spreadable formulation than other formulations.
- Irritancy test: the formulation F4 & F5 shows no redness edema, inflammation and irritation

during irritancy studies. These formulations are safe for skin. (Table1)

- Dye test: This dye conforms that all formulations were o/w types of emulsion cream.
- Homogeneity: All formulations produce uniform distribution of extract in cream this was confirmed by visual appearance and touch.(Table2)
- Appearance: when formulation were kept in long time it found that no change in colour of cream.
- After feel:Emoliency, slipperiness and amount of residue left after application of fixed amount of cream was found. (Table2)

- Type of smear: After application of creams F4 & F5 the type of smear formed on skin were non greasy.(Table2)
- Removal: The cream F4 &F5 applied on the skin was easily removed by washing with the tap water. (Table2)

Table 13. Types	s of adverse	effect of fo	ormulations:
14010 15. 1 jpc.	or adverse		inanaciono.

Formulation	Irritant	Erythma	Edema
F1	-	-	-
F2	-	-	-
F3	-	-	-
F5	-	-	-
F5	-	-	-

Table 14. Physical parameter of F4 and F5cream on room is accel	lerated temperature.
---	----------------------

Days	Temperature	Formulations	Parameter	Parameter					
			pH X1	X2	X3	X4	X5	X6	
0	RT	F4	6.7 ++	NCC	++	Е	NG	E5	
		F5	6.6 ++	NCC	++	Е	NG	E5	
	+0c+1c	F4	6.5 ++	NCC	++	Е	NG	ES	
		F5	6.5 ++	NCC	++	Е	NG	ES	
5	RT	F4	6.6 ++	NCC	++	Е	NG	E5	
		F5	6+ ++	NCC	++	Е	NG	E5	
	+0C+1C	F4	6+ ++	NCC	++	Е	NG	E5	
		F5	6.3 ++	NCC	++	Е	NG	E5	
10	RT	F4	6.6 ++	NCC	++	Е	NG	E5	
		F5	6.4 ++	NCC	++	Е	NG	E5	
	+0C+1C	F4	6.6 ++	NCC	++	E	NG	E5	
		F5	6.5 ++	NCC	++	E	NG	E5	
15	RT	F4	6.4 ++	NCC	++	Е	NG	E5	
		F5	6.5 ++	NCC	++	Е	NG	E5	
	+0C+1C	F4	6.7 ++	NCC	++	Е	NG	E5	
		F5	6.3 ++	NCC	++	E	NG	E5	
20	RT	F4	6.5 ++	NCC	++	E	NG	E5	
		F5	6.6 ++	NCC	++	Е	NG	E5	
	+0C+1C	F4	6.4 ++	NCC	++	E	NG	E5	
		F5	6.3 ++	NCC	++	E	NG	E5	

- X1: Homogeniety, X2: Appearance, X3: Spredability, X4: After feel, X5: Type of smear, X6: Removal,
- ++: Good, +: Satisfactory, E: Emollient, NG: Non-greasy, NS: Easy, NCC: No change in colour.

III. DISCUSSION

The herbal foot cream was prepared using the extract of aloevera plant shows good antimicrobial test against S. aureus, found to be maximum with the formula 5 from antimicrobial evaluation. It is found that the crack cream prepared using aloe extract is found to be effective against S.aureus which is mainly responsible for foot disorders. From subjective evaluation, it is found that the foot cream is having good appearance, spreadability and provide necessary protection against infection and provide good healing property for cracked heels.

CONCLUSION

From the above discussion, we conclude that the herbal crack cream prepared from aloe extract and ghee are acceptable in view of appearance spreadability and antimicrobial activity. The herbal crack cream is effective against S.aureus and shows good healing property for cracked heels.

These suggest again that the composition of extract and bases of cream F4 &F5 are more stable and safe, but it may produce synergistic action.

REFERENCES

- A. Dhase, S. Khadabadi and S. Saboo 'Formulation and evaluation of Vanishing Herbal cream of crudedrug' American Journal of Ethnomedicine, Vol. 1, Page no. 5, 313-318.
- Wilkinso J.B., Moore R.J; 1982, cosmeticology; 7th edition; George Godwin Publication, Page no. 191-192.
- [3] Kritikar K.R., Basu B.D.; 1981; Indian Medicinal Plants; 2nd edition; International Book Distributors; Vol. 3, Page no. 2318.
- [4] https://amoge.com/blogs/problems&solutions.
- [5] https://www.foot&ankledoctors.com
- [6] Aloevera, properties & value added products; by M.M. Azam, D.V. Singh, S. Kumar, A. Kumar, M.M. Roy, Central Arid Zone Research Institute, Jodhpur/ www.cazri.res.in
- [7] https://en.wikipedia.org/wiki/aloe_vera
- [8] ^{^abcdef} "Aloevera (true aloe)" cab 1. 15 February 2019 Retrieved 15 october, 2019.
- [9] ^{^abc}Yates A. (2002) Yates Garden Guide Harper Collins, Australia.

- [10] ^{^abcd}Random House Australia Botanicals Pocket
 Gardening Encyclopedia for Australian
 Gardeners, Random House Publishers,
 Australia.
- [11] [^]Gong M., Wang F., Chen Y., (2002). "[Study on application of arbusenlar-mycorrhizas in growing seedins of Aloevera.]"
- [12] Pawar A., Gaud R.S., 'Modern Dispensing Pharmacy', Second edition, Career Publicatiom, April 2005, Page no. 227.
- [13] https://www.researchgate.net
- [14] A. Rajvanshi, S. Sharma, S.L. Khokra, R.K. Sahu, R. Jangde, Pharmacologyonline, 2011, 2, Page no. 1238-1244.
- [15] "Textbook of Microbiology" by M.J. Pelczar, J.R.E.C.S Chain, Noc/ R.K. Poing, Fifth edition.