"FORMULATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF POLYHERB GEL"

Submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

BY

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ACADEMIC YEAR: 2019-2020

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This is to certify that the project entitled **Formulation and Evaluation of Antimicrobial Activity of Polyherb Gel** is a bonafide work of **Sarguru Rimsha (Roll No.:16ph42), Shaikh Munazza (Roll No.:16ph54), Hawai Owais (Roll No.:15ph09) submitted** for the appreciation of the degree of Bachelor of Pharmacy in Department of Pharmacology.

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ACKNOWLEDGMENT

I would like to take the opportunity to express my sincere thanks to our guide **Saba Shaikh**, Assistant Professor, Department of Pharmacy, AIKTC, School of Pharmacy, Panvel for her invaluable support and guidance throughout our project research work. Without her kind guidance & support this was not possible.

I am grateful to her for her timely feedback which helped us track and schedule the process effectively. Her time, ideas and encouragement that she gave us helped us to complete our project efficiently.

I would also like to thank Dr. Abdul Razak Honnutagi, Director of AIKTC, Panvel, for his encouragement and for providing an outstanding academic environment, also for providing the adequate facilities.

I am thankful to Dr. Shariq Syed, Dean, School of Pharmacy, Panvel and all my B.Pharm. Teachers for providing advice and valuable guidance.

I also extend my sincere thanks to all the faculty members and the non-teaching staff and friends for their cooperation.

Last but not the least, I am thankful to all my family members whose constant support and encouragement in every aspect helped me to complete my project.

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DECLARATION

We declare that this written submission represents our ideas in our own words and where others ideas or words have been included, we have adequately cited and referenced the original sources. We also declare that we have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in our submission. I understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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KEYWORDS AND GLOSSARY

1. **Zoonotic diseases-** It is an infectious disease that passes from an animal or insect to a human.
2. **Opportunistic infection-** Caused by pathogens (bacteria, viruses, fungi or protozoa) that take advantage of an opportunity not normally available, such as a host with a weakened immune system, an altered microbiota, or breached integumentary barriers.
3. **Persistent infection-** It is characterized as those in which the virus is not cleared but remains in specific cells of infected individuals.
4. **Synergistic activity-** It is an additive property of individual's agents.
5. **Bacteriostatic-** Biological or chemical agents that stops bacteria from reproducing.
6. **Incubation-** The period over which eggs, cells etc are incubated.
7. **Phytochemical study-** It is refers to the extraction, screening and identification of the medicinally active substances found in plants.
8. **Broth-** A liquid containing proteins and other nutrients for the culture of bacteria.
9. **Inoculum-** Material used for inoculations.
10. **Zone of inhibition-** Is a circular area around the spot of antibiotic in which the bacterial colonies do not grow.
11. **Maceration-** Is the process by which organized tissue is transformed into a suspension if intact cells.

1. INTRODUCTION:

The practice of herbal medicine has existed since prehistoric times as the primary form of medicine. In this space age where the technology has very much advanced, herbal medicines still flourish and are finding exceptional acceptance in both the developing and the developed countries due to their natural origin and lesser side effects. Besides widespread use of botanicals as medicinal products in developing countries, such products are fast becoming a part of the integrative healthcare systems of the industrialized nations, known as complementary and alternative systems of medicine.

A number of herbal traditions have come to dominate the practice of alternative medicine. These include the western herbal tradition based on Greek, Roman and medieval sources, the essentially Ayurvedic tradition of India and the Chinese herbal medicine. The traditional Chinese medicine continues as a distinct branch of modern medical practice .The traditional herbal remedies as alternative medicine plays a significant role in South Africa also, where it forms a part of the culture and beliefs of the indigenous population and also features significantly in primary health care. Botanicals or phytomedicines have always been a major component of traditional systems of healing in developing countries, which have also been an integral part of their history and culture. In the ancient Indian system of medicine, Ayurveda and Siddha are such examples [1].

It was well known to the ancient world that plants are a rich source of a variety of chemicals with nutritive and therapeutics properties. Herbs belong to general botanicals of various types which are also often the aromatic plants used especially in medicine or as seasoning. Herbs may be used directly as teas or extracts and they may be used in the production of drugs. A drug or preparation made from a plant or plants and used for any of such purpose is better known as herbal drug.

Many of the pharmaceuticals currently available have a long history of use as herbal remedies including opium, aspirin, digitalis and quinine. While purification and quantification of these plant extracts makes them more predictable and chemical processing can sometimes modify their effects in desirable ways, herbal remedies tend to have a more complex and subtle mix of chemicals, and can sometimes offer access to drugs or combinations of drugs, that the pharmaceutical industry has not yet exploited.

The widespread use of herbs in traditional medicine has also prompted demands that herbal remedies has

been regulated as drugs to ensure quality standards and to prove its scientific basis. Herbs hold promise not only for prevention but also for the treatment of various types of diseases. The drugs of natural origin constitute very important and valuable segments of modern medicine. Traditional medical practitioners and scientists are turning towards medicinal plants for curing ailments such as inflammation, rheumatoid arthritis, cancer, diabetes and many more because of the fact that they possess lesser side effects owing to their natural origin. These extracts are formulated into different formulations for ease of administration. The novel formulations are reported to have remarkable advantages over conventional formulations of plant actives and extracts which include enhancement of solubility, bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improved tissue macrophages distribution, sustained delivery, and protection from physical and chemical degradation [2].

WHAT IS INFECTIOUS DISEASE?

Infectious diseases are diseases caused by living organisms like viruses and bacteria. They can be passed from person to person through body secretions, insects or other means. Examples are SARS, microbial and bacterial infection, influenza, and common cold, tuberculosis (TB), Hepatitis A and B.

INFECTION according to WHO:

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi; the diseases can be spread, directly or indirectly, from one person to another. Zoonotic diseases are infectious diseases of animals that can cause disease when transmitted to humans [3].

WHAT IS ANTIMICROBIAL AGENTS

Microbes are microscopic organism that includes any unicellular, cell bunches, or a cellular [4], found nearly everywhere in the taxonomic society of life on the sphere. In modern epoch, medical science along with other different challenges also stand in front of a challenge of escalating microbial resistance against different antibiotic [5] due to which now at present, researchers focuses on antimicrobial activity of traditional plants to overcome the microbial resistant pattern [6-10] however most of the active compound are present in these plants that can be used globally for different health care purposes [11-12]. Microbes can develop beneficial endosymbiotic association with other organisms in spite of causing of many contagious infections. These microbes include pathogenic bacteria (as plague, tuberculosis, and anthrax), protozoa (malaria, sleeping sickness, toxoplasmosis), fungi (ringworm, candidiasis, histoplasmosis). Skin

floras are habitually non-pathogenic or mutualistic microorganisms (mostly 1000 species of bacteria) exist on skin, mostly on superficial or upper layers of the epidermis and hair follicles [13-15]. The non-pathogenic bacteria can deal with avoiding transitory pathogenic organisms from colonizing the skin superficial. Though, the occupant organism on skin can cause infection and enter the blood system producing life-threatening infections mainly in immune compromise person [16].

Different leaf part of plant has been used since ancient time either extracted raw compound or make a paste [17]. Although several plant species have been evaluated as a best choice for antimicrobial activity [18-21]. As these plants contain such an essential component which is consider to be having an antimicrobial effect [22-25].

Multiple ranges of self-limiting fatal infections in humans are caused by S. aureus which is an opportunistic pathogen. Usually neonates and young children have scale skin syndrome which is due to exfoliative toxins produce on epidermis [26]. Similarly blisters, pimples, boils, impetigo, folliculitis and other skin disorders like abscesses and skin loss are due to exfoliative toxin produce by Staphylococcus species [27-29]. Immuno-suppressive people have necrotizing fasciitis which is due to *S. aureus* though this condition is rare [30].

Many factors contribute to the ability of pathogens to establish persistent infections, including both host and bacterial factors. Certain pathogens appear uniquely adapted to evade the host immune system and persist in infected individuals for decades in the absence of symptoms, for example *Mycobacterium tuberculosis* or *Salmonella* Typhi [31-32].Other pathogens like *Pseudomonas aeruginosa* or *Escherichia coli* can cause both symptomatic acute and chronic infections, with specific changes in the host facilitating the establishment of a persistent infection. A key element of this different physiologic state is a non-replicating or slowly replicating growth rate, which may have the additional benefit of contributing to a pathogen's defence against antibiotic exposure. Walsh McDermott first suggested in the 1950s that the relative metabolic state of bacteria affects antibiotic efficacy, causing cells to become "indifferent" to antibiotics, thereby relating the physiologic state of bacteria to antibiotic efficacy [33].

During present study, following two plants namely, *Hibiscus sabdariffa L.* and *Amaranthus cruentus L.* were used to evaluate their antimicrobial activity. These plants were selected for the study because of their excessive usage in daily diet. A number of researches have been already done on these plants but there

isn't much work done on its combinational antimicrobial activity. These plants are supposed to be very effective against different microbes. Therefore this study is made to compare their synergistic antimicrobial activities of the selected two plants with that of the standard drug.

Ambadi is a communal name for the herb *hibiscus sabdariffa L.* belong to Malvaceae family and Laal chawli is a communal name for *Amaranthus cruentus L.* belonging to Amaranthaceae family. Various *in vitro* scientific studies proved that the above two plants have strong antioxidant, hepatoprotective, antimicrobial and antibacterial properties

Figure 1: *Hibiscus sabdariffa L.*

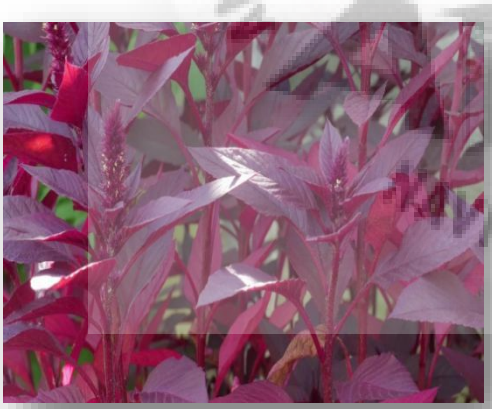

Figure 2: *Amaranthus cruentus L.*

2. REVIEW OF LITERATURE

Synergy between antimicrobial agents means that, when studied in vitro, the combined effect of the agents is greater than the sum of their independent activities when measured separately [34]. For example, the combination of certain β-lactams and aminoglycosides exhibits synergistic activity against a variety of gram-positive and gram-negative bacteria [35] and is used in the treatment of serious infections, for which rapid killing is essential (eg, treatment of endocarditis caused by *Enterococcus* species with a combination of penicillin and gentamicin). In this setting, the addition of gentamicin to penicillin has been shown to be bactericidal, whereas penicillin alone is only bacteriostatic and gentamicin alone has no significant activity. For certain streptococci, similar synergistic combinations that result in more rapid clearance of the infecting microorganism can also be used to shorten the course of antimicrobial therapy (eg, for endocarditis due to viridans group streptococci, a combination of penicillin or ceftriaxone with gentamicin for 2 weeks can be as effective as penicillin or ceftriaxone alone for 4 weeks [36-37].

Invitro Antimicrobial Evaluation:

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. In this review, we focused on the use of antimicrobial testing methods for the *in vitro* investigation of extracts and pure drugs as potential antimicrobial agents.

Natural products are still one of the major sources of new drug molecules today. They are derived from prokaryotic bacteria, eukaryotic microorganisms, plants and various animal organisms. Microbial and plant products occupy the major part of the antimicrobial compounds discovered till now [38].

Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on investigation of plant and their microbial extracts, essential oils, pure secondary metabolites and new synthetized molecules as potential antimicrobial agents [39-41]. However, when we reviewed the published articles on the antimicrobial effect of the natural products, the comparison between results is often difficult, because of the use of different non-standardized approaches inoculum preparation techniques, inoculum size, growth medium, incubation conditions and endpoints determination.

The fact that a plant extract exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to compare results, avoiding work in which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study.

A variety of laboratory methods can be used to evaluate or screen the *in vitro* antimicrobial activity of the prepared extract and pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods.

Owing to the new attraction to the properties of new antimicrobial products like combating multidrug-resistant bacteria, it is important to develop a better understanding of the current methods available for screening and/or quantifying the antimicrobial effect of an extract or a pure compound for its applications in human health, agriculture and environment. Therefore, in this review, the techniques for evaluating the *in vitro* antimicrobial activity were discussed alongwith other parameters.

1. Agar disk-diffusion method:

Agar disk-diffusion testing was developed in 1940 [42], is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing [43-44].

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism (*Escherichia coli, Staphylococcus aureus)*. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The inoculated petri plates were incubated at 37°C and examined after 24 hours for inhibition zones around the wells. Zones were measured in mm by a ruler for each disc exhibiting inhibition zones under and around discs.

Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.

Disk-diffusion assay offers many **advantages** over other methods:

- Simplicity
- Low cost
- The ability to test enormous numbers of microorganisms and antimicrobial agents

- The ease to interpret results provided.

Moreover, several studies have demonstrated the great interest in patients who suffer from bacterial infection caused by different causative agent [45]. This fact is due to the good correlation between the *in vitro* data and the *in vivo* evolution [46].

Currently, a standardized amicrobial disk-diffusion approach is used to test the synergistic effect of combination of two herbal extract.

The above-mentioned advantages of this method, mainly simplicity and low cost, have contributed to its common use for the antimicrobial screening of plant extracts, essential oils and other drugs [47-51].

During the literature review we found that there were many reported activity that have been done on these two plants. They are as follows-

Reported activity of *Hibiscus sabdariffa*-

1. Puri D.et al(1994)
   Studied In East Africa, hot water extract of Hibiscus sabdariffa linn leaves is taken orally to relieve coughs. Unripe fruit juice is taken orally with salt, pepper, as a fecide and molasses as a remedy for biliousness.

2. Bajpai H.S.et al(1978)
   Studied H. sabdariffa In Egypt, decoction of hot water extract of the calyx is taken with sugar three times daily for high blood pressure.

3. Tiwari K.A,(2001)
   Studied multiple approach of natural antioxidants therapy in imbalance in anti-oxidant defence and human diseases.

4. Michele L.D et al (2007)
   Investigated the efficacy and safety of H. sabdariffa for treating obesity.

5. Morton J.F,(1974)
   Studied the different parts of the Hibiscus sabdariffa plants.

6. Bouquet A.et al,(1969)

   Reviewed dried calyx at a concentration of 10.0%of diet in the ration of rat, and also reported that dried calyx at a concentration of 5.0% of diet in the ration of rats, showed the antihypercholesterolemic, antihyperlipidemic and antihypertriglyceridemia activity.

7. BAKO I.G,et al(2009)

   Investigated the antioxidant activity of ethanolic seed extract of H.Sabdariffa in sodium nitrate toxity indused wistar rat.

8. Hatil Hashim,et al(2006)

   Evaluated the antibiotic effect of Hibiscus sabdariffa, E- coli showed higher resistance to the plant extracts whereas Pseudomonas aeruginosa is more sensitive.

9. Hala H. Mossalam,et a l(2011)

   Evaluated that aqueous extracts of Hibiscus sabdariffa possess a potent protective effect from the oxidative stress induced by sub lethal dose of Malathion on the kidney.

10. Ismaila A. Umar,et al,(2009)

    Investigated the pathological changes in blood and organs of T.congolense-infected rats.

11. Deependra Soni,et al (2011)

    Investigated the experimental work on root part, the presence of various phytoconstituents like flavonoids, tannins, protein, sterol etc.

12. Agbafor K.H,et al,(2011)

    Investigated the antioxidant potential of extracts and some of the therapeutic uses of these plants.

13. Saleh A.et al,(2010)

    Investigated the gastroprotective effects of ethanolic extract of the calyces of H. sabdariffa (EEHS).

14. Titilayo O. Fakeye et al,(2008)
    Evaluated the toxic effect of oral administration of extract of dried calyx of H.sabdariffa.

15. Yadong Q.et al(2005)
    Studied the biological characteristics, food use, and medicinal values of Roselle, Hibiscus sabdariffa.

16. Hanumanthacher Joshi et al,(2006)
    Investigated the aqueous extract of calyces of H. sabdariffa might prove to be a useful memory restorative agent in the treatment of dementia seen in elderly.

17. Hambrook,(2009),
    US20090232786,A1, Invented herbal formulation for cancer, it predicted in identification and combination of number of plant or there parts which are usefull in treatment or prophylaxis of cancer or inflammation.

18. Vanata R.et al(2007)
    Evaluated the antipyretic activity of the extract of the H.sabdariffa calyx on noceptive response.

Reported activity of *Amaranthus cruentus*-

1) Iron chelating activity obtained in methanol and hexane extract of A. cruentus leaves were 64% and 54% respectively; [52].

2) Antioxidant potential has been attributed to the presence of appreciable levels of phenolics and flavonoids. Leaves and flowers of *Amaranthus* as well as their extracts were shown to possess highest antioxidant activities compared to other parts; rutin being the major radical-scavenger [53].

3) A study based onhydroacetonic, methanolic and aqueous extracts prepared fromaerial parts of A. cruentus and A. hybridus; conducted by Nanaet al. [54] described these extracts as having antioxidant and xanthine oxidase inhibitory activities in vitro.

4) Gandhi et al. [55] tested the antiproliferative activity of A. cruentus aqueous extract on humanperipheral lymphocytes and suggested that it could be used as aninexpensive, biocompatible, commercial alternate to availableanti-proliferative therapeutics.

5) Escudero et al. [56] based on their observations of lipid profile and liverhistoarchitecture in Wistar rats, concluded that presence of phenols in flour and protein concentrate of A. cruentus seeds provokesan increase in antioxidant defenses, thus playing a protective rolein liver.

6) In a study by Islam et al. [57], chloroform leaf extracts of A. viridis showed antibacterial activityagainst Bordetella bronchisseptica, Bacillus pumilus, Staphylococcusaureus and Proteus vulgaris.

3. AIM AND OBJECTIVES:

AIM

The aim of present study is to find out synergistic activity of two plant.

OBJECTIVE

1. To find out antimicrobial activity of aqueous extract of leaf of *Hibiscus sabdariffa*
2. To find out antimicrobial activity of aqueous extract of leaf of *Amaranthus cruentus*
3. To Formulate polyherb gel
4. To find out antimicrobial activity of polyherb gel
5. To evaluate of polyherb gel

4. EXPERIMENTAL WORK:

Plan of work

Collection of plant

The plant material was collected from the local vegetable market of Panvel, Navi Mumbai in the month of September and authenticated from the Botany Department, khalsa college College Mumbai. (Specimen#: sps p 0120204061 and Specimen #: sps p 0120204009). Freshly collected leaves of *Hibiscus sabdariffa* and *Amaranthus cruentus* were dried under shade. Dried leaves were ground to a coarse powder with an electrical blender.

Authentication of plant-

The plant material was collected from the local vegetable market of Panvel, Navi Mumbai in the month of September and authenticated from the Botany Department, Khalsa College Mumbai. (Specimen#: sps p 0120204061 and Specimen #: sps p 0120204009).

Pharmacognostic study

Microscopical characteristics of powdered drug and TS of leaf **(**For both *Hibiscus sabdariffa* and *Amaranthus cruentus***)**

Take 1-2gm of leaf powder in a test tube and add 1ml of chloral hydrate solution. Heat on a bunsen burner for 10-15 minutes. Boil it gently. Transfer the mixture onto the watch glass. With the help of brush take required quantity of sample on a micro slide. Put the cover slip and observe under the microscope.[58]

Preparation of extract-

Two different Plants were dried, powdered and extracted with distilled water. For experimental work, two plant aqueous extracts were prepared by maceration method. 500 g of the drug were weighed and separately it was mixed with water. The whole process was kept in dark conditions for seven days with frequent shaking every 2 hours. After seven days Anthe extract was filtered and the filtrate was concentrated by distilling of the solvent till one third. After that the extract was concentrated over water bath at a temperature not exceeding 60^{o}C. The extract was further dried over desicator for overnight and was used further for the experiments [59].

Gel preparation

In this study we prepared 1% w/w gel formulations, which comprised of aqueous extract of *Hibiscus sabdariffa* and *Amaranthus cruentus* in the ratio of 50:50, respectively in a base. The base was prepared by using carbapol 940, propylene glycol-400, ethanol, methyl paraben, propylparaben, EDTA, triethanolamine and required amount of water in a quantity sufficient to prepare 10g [60].

The microorganism and growth medium

Staphylococcus aureus (MTCC 96) and *Escherichia coli* (MTCC 443) were chosen based on their clinical and pharmacological importance [61]. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Antimicrobial activity assay

The *In vitro* antimicrobial activity was performed using disk diffusion method [62]. First the antimicrobial activity of aqueous extract of *Hibiscus sabdariffa* and *Amaranthus cruentus* were done to check synergistic effect followed by determination of antimicrobial activity of formulated polyherb gel.

Evaluation of gel:

1. **pH:** pH of individual and polyherbal gel formulation was determined by using a pH meter (Table 3).
2. **Appearance and Homogeneity:** The developed individual and polyherbal gels were evaluated for physical appearance and homogeneity by visual observation (Table 3).
3. **Viscosity:** The viscosity of individual and polyherbal gels was measured by Brookfield viscometer (Model RVTDV II) at 100 rpm using spindle no. 6 (Table 3).
4. **Spreadability:** The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after one min. The standard weight applied on the upper plate was 125 gm (Table 3).
5. **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. 0.5 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)

5. RESULTS:

5.1 PHARMACOGNOSTIC EVALUATION OF PLANT

5.1. A. Pharmacognostic Characteristics of *Hibiscus sabdariffa:*

- **T.S of *Hibiscus sabdariffa***

Microscopy of leaf: Dorsal surface

Figure 3: T.S of *Hibiscus sabdariffa*

MIDRIB-

i. **Epidermis:** Epidermal layers are continuous.
ii. **Collenchyma:** Below,upper and lower epidermis.
iii. **Vascular bundles:** (Xylem)- Lignified
(Phloem)- Non lignified

LAMINA-

iv. **Upper epidermis:** Straight walls, single layered.
v. **Trichomes:** Covering
vi. **Stomata:** Cruciferous

MESOPHYLL-

vii. **Palisade:** single layered.
viii. **Spongy parenchyma:** 6-8 layers, loosely arranged, intercellular spaces, cluster crystals.

LOWER EPIDERMIS-
Similar to upper epidermis, stomata and numerous trichome

- **Powdered Characteristics of *Hibiscus sabdariffa:***

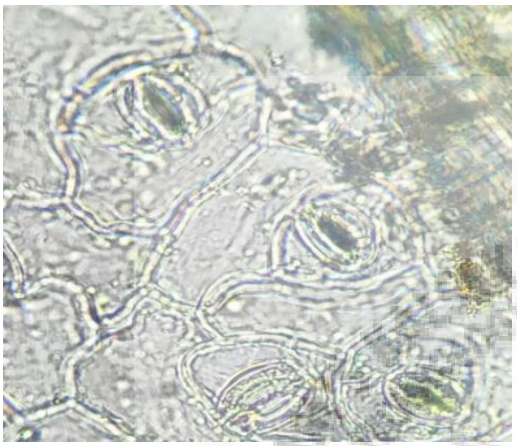

Figure 4- Stomata:
Cruciferous stomata(anisocytic)

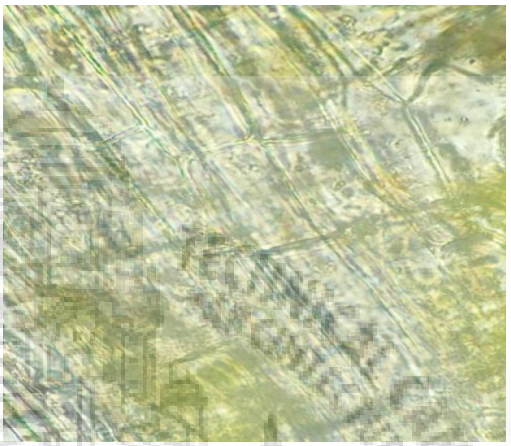

Figure 5- Epidermal cells:
Slightly straight in upper epidermis.

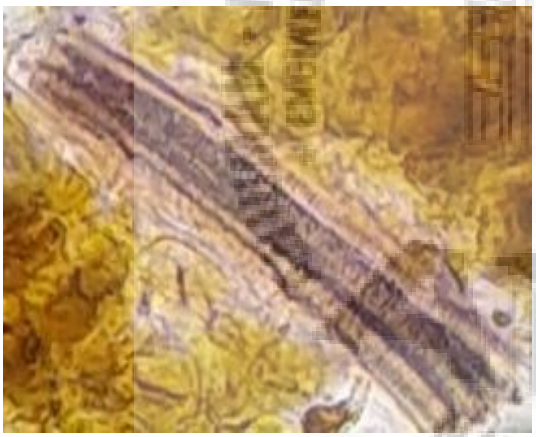

Figure 6-Xylem vessel:
Pitted and lignified.

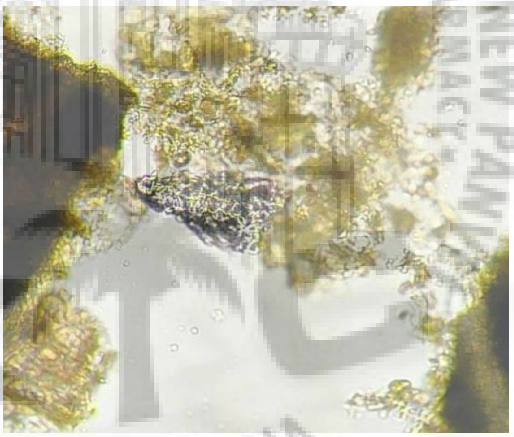

Figure 7-Trichomes:
Occur in spongy Parenchymatous cells and scattered in the powder.

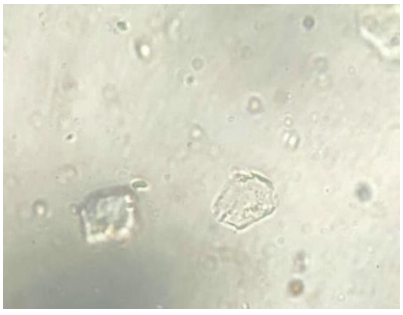

Figure 8- Calcium Oxalate crystal

5.1. B. Pharmacognostic Characteristics of *Amaranthus cruentus:*

- **T.S of *Amaranthus cruentus***

Microscopy of leaf: Dorsal surface

Figure 9: T.S of *Amaranthus cruentus*

MIDRIB:

(i)Epidermis: Epidermal layers are flat and continuous.

(ii)Collenchyma: Found at upper and lower epidermis.

(iii)Vascular bundle: (Xylem): Lignified and pitted.

(Phloem): Lignified.

LAMINA:

(iv)Upper epidermis: Single layer,flat.

(v)Trichomes: Coverings.

(vi)Stomata: Cruciferous.

MESOPHYLL:

(vii)Palisade:

LOWER EPIDERMIS:

Same as upper epidermis,stomata and trichomes

- **Microscopical characteristics of *Amaranthus cruentus*:**

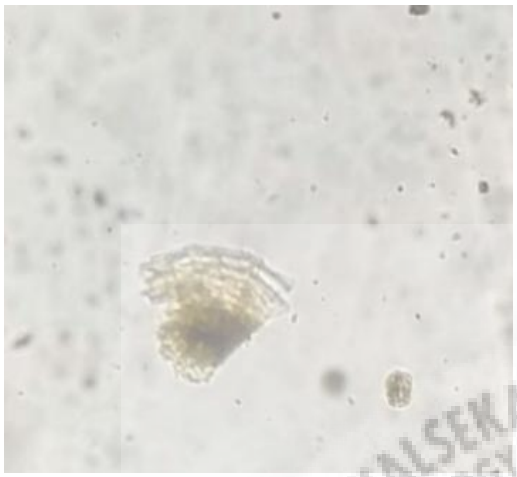

Figure 10- Epidermal cells: **Flat and continuous.**

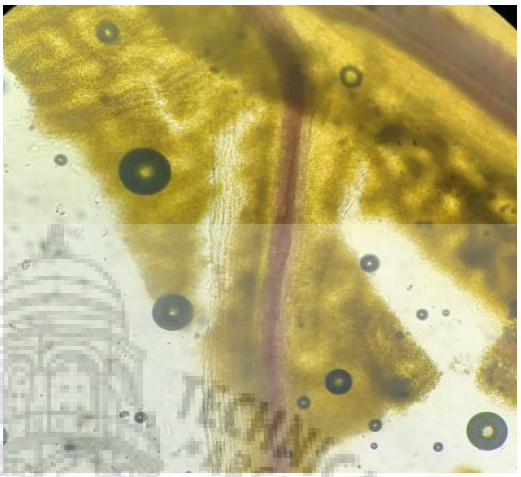

Figure 11- Xylem vessel: **Pitted.**

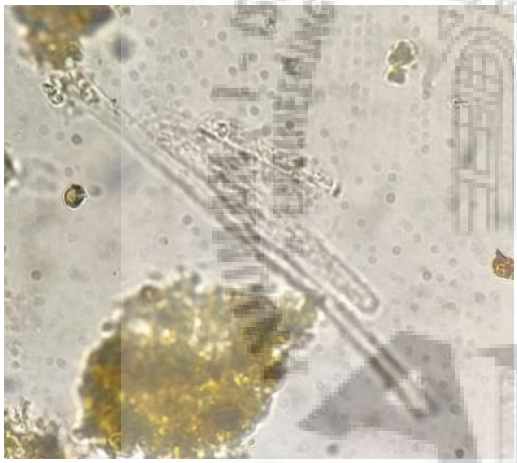

Figure 12- Trichomes: **Occur in spongy parenchymatous cells.**

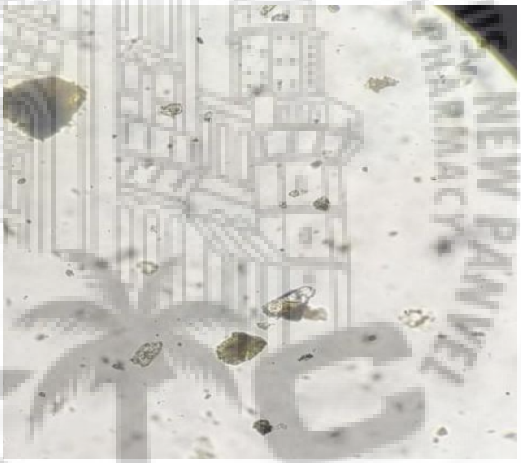

Figure 13- Calcium oxalate crystals: **Scattered In powdered drug.**

5.2 ANTIMICROBIAL ACTIVITY OF EXTRACT FOR SYNERGISTIC PROPERTY:

Microorganism	**Antimicrobial activity**			
	Zone of Inhibition (mm)			
	Standard (Ciprofloxacin) (I)	Aqueous extract of *Amaranthus cruentus* (II)	Aqueous extract of *Hibiscus sabdariffa* (III)	*Amaranthus cruentus* +*Hibiscus sabdariffa (50:50)* (IV)
E. Coli	41	8	5	24
S. aueus	40	9	11	37

Table 1- Calculation of zone of inhibition (in mm) of extract

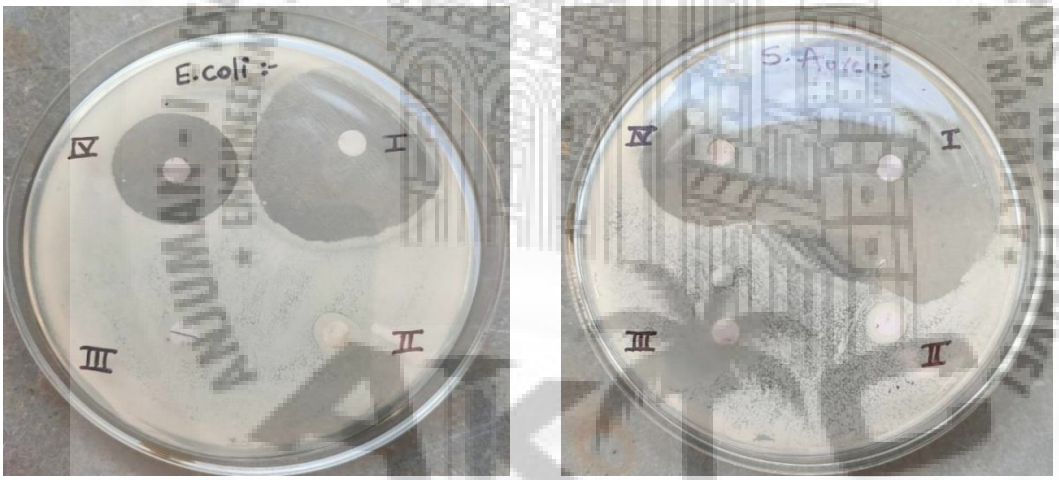

Figure 14: Synergistic antimicrobial activity of two extract

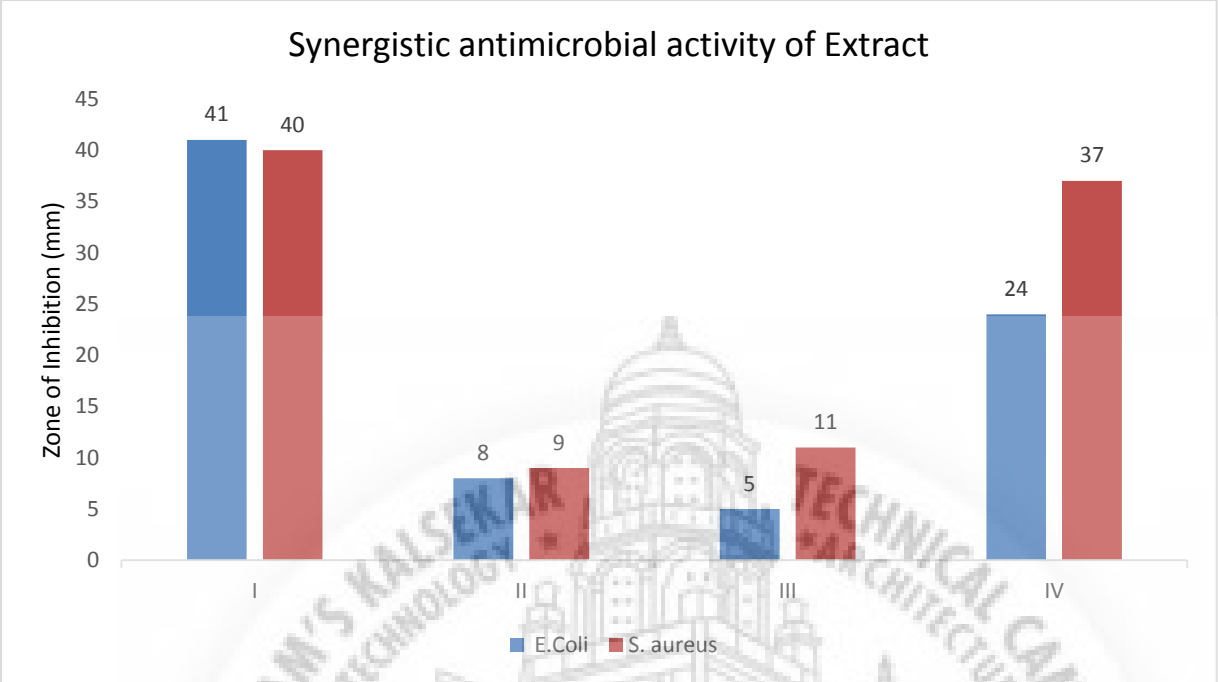

Figure15: Graph of Synergistic antimicrobial activity of two extract

5.3 ANTIMICROBIAL ACTIVITY OF FORMULATED POLYHERB GEL:

Microorganism	**Antimicrobial activity**	
	Zone of Inhibition (mm)	
	Standard (Clotrimazole Gel) Candid Gel	Polyherb Gel
E. Coli	9	4
S. aueus	9	8

Table 2-Calculation of zone of inhibition (mm) of the formulation

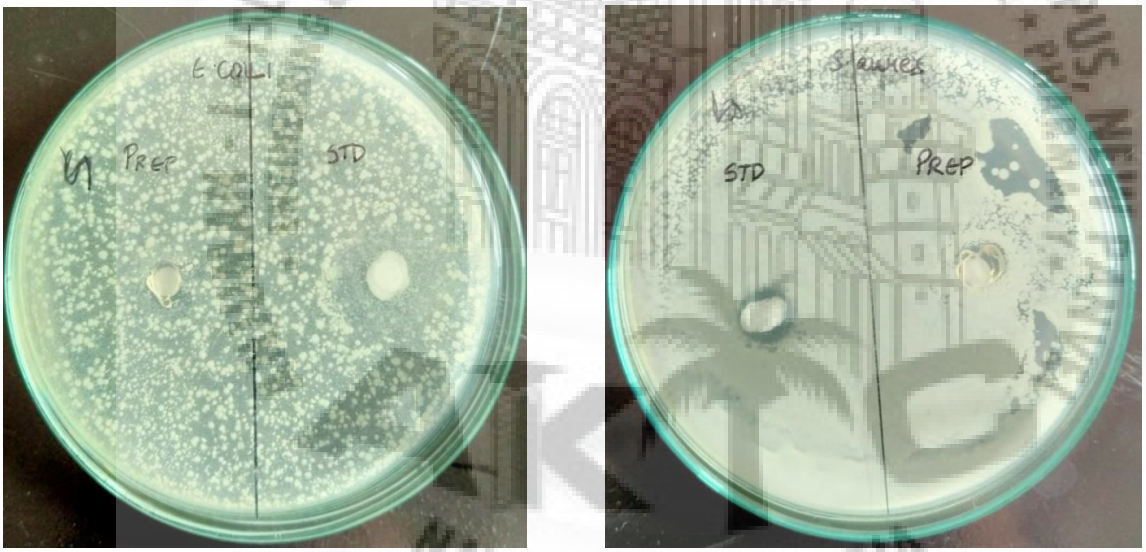

Figure 16: Antimicrobial activity of polyherb gel

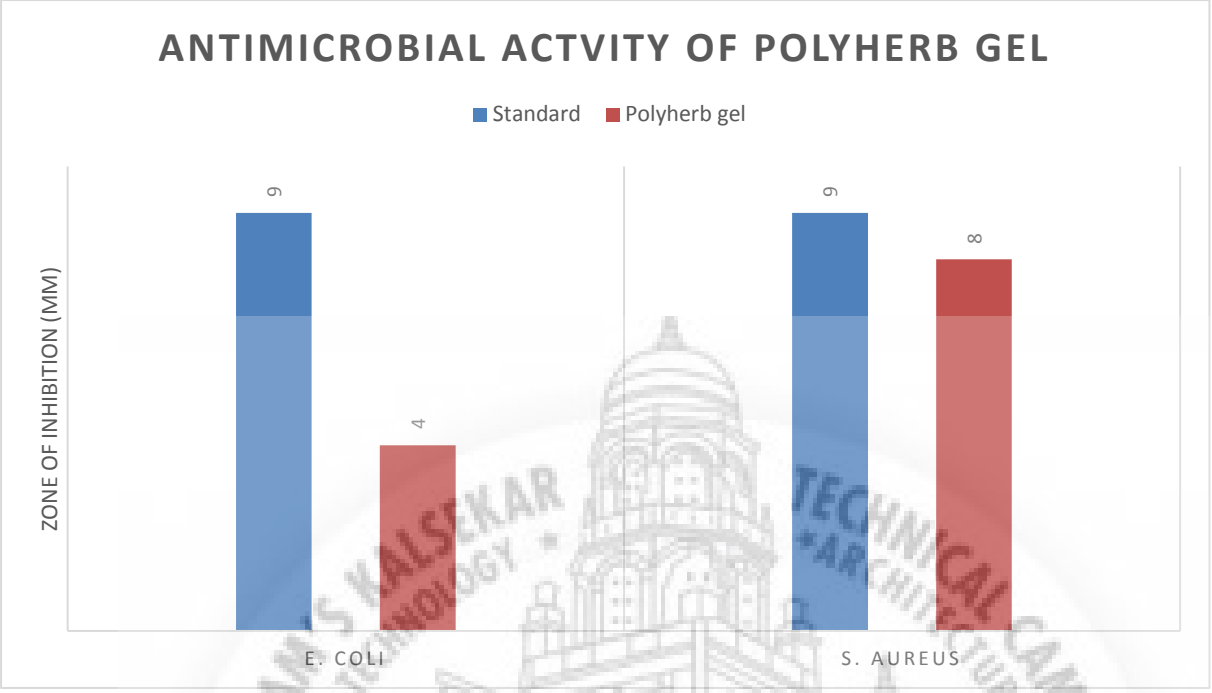

Figure 17: Graph of Antimicrobial activity of polyherb gel

5.4 PHARMACEUTICAL EVALUATION OF POLYHERB GEL:

Parameters	Polyherb gel	Marketed gel
pH	6.45	6.13
Appearance	Light brown	White
Homogeneity	Good	Good
Viscosity (cp)	42000	45000
Spreadability (spreading after 1 min)	39	56
Extrudability	Good	Excellent

Table 3: Pharmaceutical evaluation of Polyherb Gel

6. DISCUSSION:

Herbal drugs are getting popularity and its pharmacological properties are reported from different part of the world. In the current study, the aqueous extract of both *Hibiscus sabdariffa* and *Amaranthus cruentus* showed good antimicrobial activity but synergistic antimicrobial activity observed when aqueous extract of *Hibiscus sabdariffa*was used incombination of aqueous extract of *Amaranthus cruentus.* This study also include subsequent formulation of polyherb gel and the gel also showed good antimicrobial activity when compared with marketed formulation.

6. CONCLUSION

As per the result obtained, it could conclude that this polyherb gel possesses antimicrobial activity. Thus can be used in the treatment of topical infectious disease. The current study also appreciate synergistic antimicrobial activity of vegetables like *Hibiscus sabdariffa* and *Amaranthus cruentus* which are widely used in Maharashtra region. Overall results of current study demonstrate that polyherb gel has good antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

8. FUTURE SCOPE:

The future scope we can drive from the above research is that we can carry out more activity which are not reported till date and to carry out the preclinical study of polyherb gel.

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10. APPENDIX

AUTHENTICATION LETTER-

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January 19th, 2020

Ms. Saba P. Shaikh
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Plot No. 2 & 3, Sector-16, Near Thana Naka
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Hibiscus sabdariffa Linn.

This is with reference to fresh material originating from Vasai market brought in by you for identification. The twigs have stem purple red in colour leaves, root and flower buds. The leaves are various types simple to 3-5 lobed, lobes lanceolate, middle lobe longest, margin serrate, glandular petiole purple red coloured. Internally the epidermis is single layered with trichomes on upper, mesophyll differentiated in upper compact palisade and lower loosely arranged spongy, druses in the leaf tissue, petiole has upto 8 vascular bundles endarch.The material (**specimen #: sps p 0120204061**) represent the leave of ***Hibiscus sabdariffa*** Linn. of family Malvaceae.
It is commonly known as Lalambadi,Ambasthika, Red sorrel, Roselle.

Yours sincerely

Harshad M. Pandit (14909/85)

1. Material 2.Single twig 3.Leaves apical bud 4. Various leaves 5. Flower bud 6. Section petiole
7. Section of leaf lamina 8. Single Vascular bundle, trichome 9. Crystals (druses)
Hibiscus sabdariffa leaf characteristics

References:
http://www.theplantlist.org/tpl1.1/record/kew-2850461
http://www.tropicos.org/Name/19600047
http://apps.kew.org/herbcat/getImage.do?imageBarcode=K001114692

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January 16th, 2020

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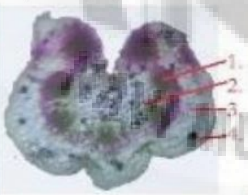
Amaranthus cruentus Linn.

This is with reference to fresh material twig of plants brought in by you for identification. The leaves are of various shapes from ovate to rhombic –ovate, apex obcordate. Their length is 7.75 cm and width 3.07 cm approximately in the given material. Internally the midrib has vascular bundles in crescent form collateral, with parenchyma and few cells with druses of calcium oxalate. The mesophyll in lamina is palisade and spongy cells with druses with single layer of epidermis and cuticle. The material (**specimen #: sps p 0120204009**) are the leaves of ***Amaranthus cruentus*** Linn. of family Amaranthaceae. It is commonly known as Rajagiri, Chaulai, Shravani math, Red or purple amaranth.

Yours sincerely

Harshad M. Pandit (14909/85)


Material twig



1. Xylem 2.Phloem
3.Parenchyma 4. Druse


Midrib and lamina


Lamina with cuticle, mesophyll, druse (d)

References:
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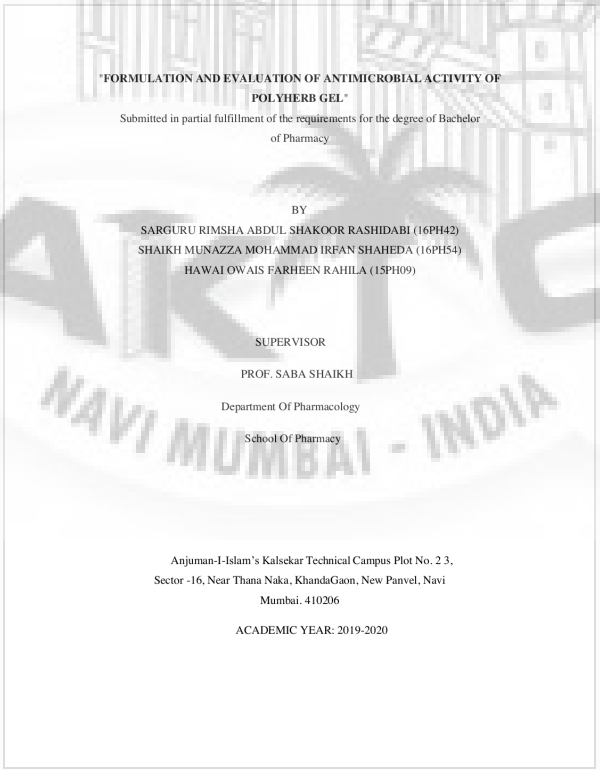

"FORMULATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF POLYHERB GEL"

Submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

BY

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