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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Evaluation of Herbal Antifungal Dermatological Formulation

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ABSTRACT
Infections caused by fungi have become a major source of morbidity and mortality. Limited number of drugs are available to treat infections. Phytochemicals can be the major source of antifungal compounds. The aim is to review the current literature to assess the challenges and scope of phytochemical research in the development of new antifungal drugs. The crude drug extract of different herbal preparations exhibits definite significant antifungal activity on most of the fungi. The various fungi include Aspergillus flavus, Candida albicans, Microsporum mentagrophytes, Trichoderma SP and Trichosporon cutaneum with respect to type of herbal extract. Literature describing cellular nature of various species of fungi for the development of drug resistance and the target sites for the new drug development. Publications reporting antifungal activity of crude extract of plant, their essential oils and identified chemical constituents of plants are reviewed. The development of new antifungal Agents from natural sources is a complex process due to its nature and type of infection caused. Efficacy of the drug in the presence of body fluids, normal flora and medical device can also pose a challenge. Synthetic, semi-synthetic and natural compounds can be screened for their antifungal activities against emerging target sites using new cost-effective techniques to increase throughput. Their efficacy, substantivity can be enhanced using nanotechnology, hydrogel formulation, and bio-adhesive technology. Conclusion: Many challenges are identified in the development of new antifungal drugs, however phytochemicals are still the major source of new antifungal drugs.
Keywords: *Antifungal agents, phytochemicals, phytomedicines, drug target, drug resistance*

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INTRODUCTION:

Pharmacognosy known initially as Materia Medica; may be defined as the scientific study of those substances, which are used or have been used in medicine and pharmacy. The word "Pharmacognosy" was coined in 1815 by seidler, a Medicine student at Halle, Germany, in his Doctoral theses. It is derived from two Greek words. "Pharmakon" meaning a drug or medicine and gignosco-meaning to acquire knowledge of. [1-2].

Hence it also has been defined as "simultaneous application of various scientific disciplines with the object of acquiring knowledge of drugs from every point of view. [3]. The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy). The development of drugs from plants continues, with drug companies engaged in large pharmacological screening of herbs.[4-5]. Its scope includes the study of the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources. Research problems in pharmacognosy include studies in the areas of Phytochemistry, microbial chemistry, biosynthesis, biotransformation, chemotaxonomy, and other biological and chemical science. [6-7].

MEDICINAL PLANTS IN INDIA

Traditional medicines are used by about 60 percent of the world population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. While the traditional medicines are derived from

medicinal plants, minerals, and organic matter, the herbal drugs are prepared from medicinal plants only. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In the Indian systems of medicine, most Practitioners formulate and dispense their own recipes; hence this requires proper documentation and research.[8-10]. In western world also, the use of herbal medicines is steadily growing with approximately 40 percent of population reporting use of herb to treat medical illnesses within the past two year. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine. There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. There are currently about 250 000 registered medical practitioners of the Ayurvedic system (total for all traditional systems: approximately 291 000), as compared to about 700,000 of the modern medicine system.[11]. In rural India, 70 percent of the population is dependent on the traditional system of medicine; the Ayurveda. The major hindrance in the amalgamation of herbal medicines into modern medical practices is the lack of scientific and clinical data, and better understanding of efficacy and safety of the herbal products. To ensure the quality and safety of its products and practices standardization is of vital importance. Most herbal products do not have drug regulatory approval to demonstrate their safety and efficacy. [12]. The traditional use can provide valuable clues for the selection, preparation and indications for use of herbal formulation, as efficacy has been established by the common use. The historical use provides the source to study the specific plant species with potential to be used in a particular disease. A systematic approach through experimental and clinical validation of efficacy is required for a plant identified for traditional medicine, as is done in modern Medicine; animal toxicity studies are also required to establish the potential adverse effects. Efficacy testing of the traditional and new herbal product in experimental screening methods is important to establish the active component and appropriate extract of the plant. However, there should be adequate data from in vivo and in vitro studies to validate the therapeutic potential claimed. There is a need to establish the pharmacological activities for identifying and comparing the various preparations for potency. [13]. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Since many of the compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patient's self- medicating with these preparations.

MATERIAL AND METHODS

PLANT PROFILE

Argyreia nervosa:

SCIENTIFIC NAME: Argyreia nervosa (Burm.f.) BojConvolvulaceae

SYNONYMS: Argyreia speciosa sweet, English : Elephant creeper, Marathi & Gujarati : Samudrasoka, Hind : Samundraka pat, Sanskrit: Samundra Palaka

Kingdom: Plantae

Order: Solanales

Family: Convolvulaceae

Genus: Argyreia

Species: A.nervosa

CHEMICAL CONSTITUENTS:

LEAF: 1-triacontanol – long chain alcohol Steroids - Epifriedelinol and its acetate, β- sitosterol, 28-pentyl-3-galloyl-betulinate, 11-hydroxy friedelane Flavonoids- Anthocyanin, Quercetin, Kaempferol and its glycosides, 3-O-rhamnopyranoside, 7,8,3',4',5'-pentahydroxyflavone, 5-O-α-D-rhamnopyranoside and 5-O-β-D- glucopyranoside.

ROOT: Tetradecanyl palmitate,5,8-oxidotetracosan-10-one Aryl esters- Stimasteryl p- hydroxycinnamate, Hexadecanyl p-hydroxycinnamate, scopoletin.

USES: Emollient, antifungal activity.

Figure 1 ***Argyreia nervosa***

ARGEMONE MEXICANA
SCIENTIFIC NAME: *Argemone mexicana*L. Family: Papaveraceae
SYNONYMS: Argemoneleiocarpa Greene; Argemoneochroleuca Sweet; EchtrustrivialisLour. Echtrusmexicanus (L.) Nieuwl.; Argemone vulgaris Spach; ArgemoneversicolorSalisb.; Argemone spinosaMoench; Argemone sexvalis Stokes; Argemone mucronata Dum. Cours. ex Steud.; Argemone mexicana var. typicaPrain;
VERNACULAR NAMES: Mexican poppy, prickly poppy, yellow thistle, Mexican thistle. Kingdom: Plantae
Order: Ranunculales Family: Papaveraceae
Genus: Argemone
Species: A.mexicana
WHOLE PLANT:berberine, protopine, sanguinarine, chelerythrine, pancorine, (+)- argentine, angoline, aronttianamide, dihydrocheilantifoline, allocryptopine, coptisine, jatrorrhizine, columbamine, oxyberberine, N-demethyl oxy sanguinarine.
LEAVES: protomexicine, mexitin,8-dimethoxy sanguinarine,13-oxoprotopine, rutin,quercetinj,eriodictyol.
USES: Antimicrobial, antifungal activity.

Figure:2 ***Argemona Mexicana***

EXPERIMENTAL WORK:
COLLECTION AND AUTHENTICATION OF PLANT MATERIALS
The plant *Argyreia nervosa* belonging to family Convolvulaceae are widely distributed throughout the tropical region of India. The second plant *Argemone mexicana*belonging to the family *Papaveraceae*is widely distributed in tropical regions of India. *Argemone mexicana*is native to Mexico and the west Indies, but has become pantropical after accidental introduction. It is naturalized in most African countries, from Cape verde east to Somalia and to South Africa. The plant *Argyreia nervosa* and *Argemone mexicana*are collected from local and rural areas of Amravati. The species for the proposed study was identified by Dr. P. W. Deotare head of Botany Department Shri Shivaji Science College, Amravati with the reference no SC/BOT/108/2016.

FOLLOWING EXPERIMENTAL WORK IS CARRIED OUT FOR THE PLANT MATERIAL COLLECTED

- Pharmacognostic evaluation
- Morphological study
- Microscopy
- Extraction of selected plant material
- Phytochemical screening
- Development of solvent system
- Development of formulation
- Evaluation of formulation

PHARMACOGNOSTIC EVALUATION

Morphology, Macroscopy: Macroscopic of plants was studied by observing the organoleptic characters such as Colour, taste, size, texture and surface characteristics.

Microscopy: In microscopical study the following procedure has been adopted

1. Leaf was cut in required size and fixed in formalin acetic acid (formalin 5ml+acetic acid 5ml+90ml ethanol)
2. After 24hrs fixing the specimens was dehydrated with graded series of tertiary butyl alcohol.
3. Infiltration of the specimen was carried out by gradual addition of paraffin wax.

Sectioning:

1. The paraffin embedded specimen was sectioned with the help of rotary microtome.
2. The thickness of section was 10-12u
3. After dewaxing the sections were stained with toluidine blue, since toluidine blue is a polychromatic stain, the staining results were remarkably good and some phytochemical reactions were obtained.
4. The dye rendered pink colour to the cellulose wall, blue to the lignified cell, dark green suberin, violet to the mucilage, blue to the protein bodies etc, wherever necessary section also stained with safranin and fast green and iodine (for starch).
5. The prepared sections were observed through the microscope and distribution of various types of tissue was noted.

EXTRACTION

EXTRACTION OF *ARGEMONE MEXICANA*

The aerial parts were dried under shade condition and made into coarse powder using a mechanical grinder. The 100gm of powdered material was initially macerated with petroleum ether for 72 hrs till the original colour of solvent reappeared with the help of Soxhlet extraction process or continuous hot percolation process the same process of hot percolation was carried out for ethanol and water as a solvent replacement. The extract was filtered and concentrated by rotary evaporator and kept in a vacuum desiccator until use. The yield of the extract was 14.34% w/w for pet ether, 10% w/w for ethanol, 18.44% w/w for water with respect to dried powder.

EXTRACTION OF *ARGYREIA NERVOSA*

The fresh leaves of *Argyreia nervosa* were collected and dried under shade and were powdered in a mechanical grinder. The dried powder was defatted with pet ether and hot percolation process was carried out till the original colour of solvent reappeared; the same soxhlation process was carried out successively for ethanol and water as a solvent. The extract obtained was filtered completely and traces of solvent were removed by using a distillation unit.

PHYTOCHEMICAL SCREENING

Plants are synthesized in biosynthetic laboratories which synthesize a lot of metabolites and they are stored in various parts. These metabolites are mainly of two types, primary and secondary metabolites. Primary metabolites such as carbohydrates, proteins and lipids are utilized as food and secondary metabolites such as glycosides, alkaloids, volatile oils, tannins etc that exerts physiological and therapeutic effects. The compounds that are responsible for medicinal properties of drugs are usually secondary metabolites. These metabolites have different chemical nature and therefore have solubility in the solvent of different polarity. Hence, plant material was subjected to successive extraction and preliminary phytochemical screening for detection of various chemical constituents. Thus, the phytochemical test was carried out.

Sr.no	Tests	Observations
1.	**Carbohydrates**	
	Molisch's test (general test) To the test tube a few drops of molisch reagent (alcoholic naphthol) was added + 2ml of conc. Sulphuric acid was added slowly from the side of the test tube.	Violet ring at the junction of two liquids showed the presence of carbohydrates.
	Tollen's phloroglucinol test Test solution +Hcl (conc)+heat+phloroglucinol	Yellow to red colour showed presence of galactose.
	Iodine test 3ml test solution + few drops of iodine solution	Blue colour appears which disappears on boiling and reappears on cooling shoes presence of non-reducing polysaccharides (starch)
	Fehling's test(reducing sugar) Fehling's solution A & B mixed in equal volume and boiled for 1 min. Equal volume of test solution was mixed & heated in boiling water bath for 5-10min.	First yellow then red ppt shows the presence of reducing sugar.
2.	**Proteins**	
	Biuret test(general test) 3ml of test solution treated with biuret reagent (4% NaOH and few drops of copper sulphate solution)	Violet or pink colour shoes show the presence of protein.
	Millon's test Treat test solution with millon's reagent and heat it on water bath	White ppt turns brick red on heating shows presence of proteins.
3.	**Fats and oils**	
	Filter paper test	Permanently stained with oil
4.	**Steroids**	
	Liebermann-burchard's reactions 2ml extract treated with chloroform+1-2ml of acetic anhydride+2 drops of conc. Sulphuric acid from the sides of the test tube	First red, then blue and finally green colour shows the presence of steroids.
	Salkowski test 2ml extract+3ml acetic anhydride. Heat & cool. Add a few drops of conc. Sulphuric acid	Blue colour appears.
5.	**Glycosides**	
	Legals test Alcoholic solution of extract+1ml pyridine+1ml sodium nitroprusside.	Pink to red colour shows the presence of cardenolides.
	Brontrager's test 3ml extract+5ml 5%$fecl_3$+5ml dil. Hcl+heat for 5 min in a boiling water bath. After cooling an equal volume of chloroform was added,shake well. To the organic solvent layer + equal volume of ammonia solution	Pinkish red colour to ammonia layer shows presence of glycosides.
6.	**Flavonoids**	
	Shinoda test Test solution in ethanol a few drops of concHcl fragments of magnesium ribbon.	Pink colour shows presence of flavonoids.
	Pew's test Test solution + zinc powder in a test tube followed by dropwise addition of conc. Hcl	Formation of purple colour or cherry colour indicates presence of flavonoids.
7.	**Alkaloids**	
	Dragendroff's reagent 3ml filtrate + few drops of Dragendroff's reagent	Orange brown precipitation shows the presence of alkaloids.
	Mayer's reagent 3ml filterate + few drops of Mayer's reagent	Cream colour shows presence of alkaloids
8.	**Tannins and phenolic compounds**	
	Lead acetate test Alcoholic solution of extract+few drops of lead acetate solution.	White precipitate shows presence of tannins and phenolic compounds.
	Bromine water test Alcoholic solution of extract +few drops of bromine water.	Decolourisation of bromine water.

Table No. 1 PHYTOCHEMICAL SCREENING

IN-VITRO ANTIFUNGAL ACTIVITY OF EXTRACTS

MATERIALS

- Microbial culture (*Candida albicans*)
- Inoculation loop, Bunsen burner
- Saline solution, Cotton swab
- Antifungal disc (fluconazole)
- Tooth pick, Incubator
- Nutrient Agar
- Potato Dextrose Agar

PROCEDURE

- Select a pure culture plate of one of the organisms to be tested. Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution.
- Repeat until the turbidity of the saline solution visually matches that of the standard turbidity.
- Take a sterile swab and dip it into the broth culture of the organism. Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab.
- Take a sterile Potato Dextrose Agar (PDA) plate and a nutrient agar (NA) plate. Use the swab with the test organism to streak a NA plate and PDA plate for a lawn of growth. After the streaking is complete, allow the plate to dry for 5 minutes.
- Antibiotic discs can be placed on the surface of the agar using sterilized forceps. Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loops.
- Carefully invert the inoculated plates and incubate for 24 hours at 37° C for Bacteria and 5-6 days at 25^0C for Fungus. After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.
- Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone.
- Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested antibiotic.

FORMULATION DEVELOPMENT

The physicochemical characteristics and function of each excipient used in this formulation development studies are described in detail in following sections.

1. Natural Aloe veragel: Fresh pulp of *Aloe vera* was extracted and use as a natural gelling agent as many active constituents present in *Aloe vera* also show an anti- fungal property in different natural cosmetics and medicines .
2. Methyl paraben:It is the methyl ester of *p*-hydroxybenzoic acid, It is used as a preservative in Formulation. Methyl paraben is an anti-fungal agent Methyl paraben is an antifungal agent often used in a variety of cosmetics and personal- care products. It is also used as a food preservative and the Methylparaben is commonly used as a fungicide in *Drosophila* food media.
3. Propyl paraben: It is a preservative typically found in many water based cosmetics such as gels, lotions, shampoos and bath products.
4. Alcohol: It is used as a sterilizer and Disinfectant
5. EDTA: Ethylene diamine tetra acetic acid (EDTA). In shampoos, gels, cleaners, and other personal care products, EDTA salts are used as a sequestering agent to improve their stability in air.
6. Triethanolamine: Triethanolamine, neutralizes fatty acids, adjusts and buffers the pH and solubilizes oils and other ingredients that are not completely soluble in water, It also serves as a pHbalancer in many different cosmetic products, ranging from cleansing creams and milks, skin lotions, eye gels, moisturizers, shampoos, shaving foams, and so on. TEA is a fairly strong base: a 1% solution has a pH of approximately 10, whereas the pH of skin is less than pH 7.

Aloe vera gel (2% w/w) and purified water were taken in a beaker and allowed to soak for 24hrs.Storred by mechanical stirrer at 400 to 650rpm. The above mentioned extracts of plants disappeared in ethanol in separate containers then added in *Aloe vera* gel. Propylene glycol 400 as penetration enhancer, methyl paraben and propyl paraben as preservative were added slowly. Then neutralized with sufficient quantity of triethanolamine with continuous gently stirring until the homogenous gel was formed. The composition of formulation F1 and F2 were prepared. The two different formulations were prepared with the help of selecting different extracts of *Argyreia nervosa* and *Argemone mexicana*in water and pet ether at different ppm concentrations (parts per million).

PHARMACEUTICAL EVALUATIONS OF GELS

All the formulated herbal gels were tested for the following parameters by the visual inspection:

Physical appearance: Appearance, Colour, Odour

Homogeneity: The homogeneity of all developed gels was checked visually for the presence of any aggregates or clumps and for appearance.

DETERMINATION OF VISCOSITY

The viscosity of formulations A,Was measured using a LV-4 Brookfield Viscometer. The viscometer was operated at 6 rpm. In order to obtain stable display reading all measurements were recorded 60 sec after the commencement of spindle rotation and a maximum of three readings were taken to obtain an average viscosity value.

EXTRUDABILITY

The formulations were filled into collapsible aluminum tubes and sealed by a crimping machine. The weight of tubes was recorded. The tubes were placed between two glass slides weight 500gm was placed on the slide and then cap was removed, the amount of extruded gel was concluded) after the gels were set in the container. The extrudability of formulation was determined as if the extrudability is >90% then it is excellent; >80% extrudability then it is good; >70 extrudability then it is fair.

THERMAL STABILITY

Most of the drug especially gels, ointments, creams emulsions may be adversely affected by variation in extreme temperature fluctuation. This type of drug product should be tested under higher temperature conditions to avoid the problem during storage and shipping. The prepared formulation was kept for 8 hours at 35-38^{o}c.

SPREADABILITY

Spreadability is a term expressed to denote the extent of area to which the topical application spreads on application to skin on affected parts. The therapeutic efficiency of formulation also depends upon its spreading value. For the determination of spreadability evaluating topical application characteristics is very important.

METHOD

For the determination of spreadability excess of sample 2gm was applied in between two glass slides and was compressed to uniform thickness by placing 100gm weight for 5min. The weight was removed and excess gel adhering to the slide was scrapped off. The two slides in the position were fixed to a stand 45^{o}angle without slightest disturbances and in such a way that only the lower side was held firmly by the opposite fangs of the clamps allowing the upper slides to slip off freely by the force of weight tied to it. 20gms of weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 5cm and separate away from the lower slide under the direction of weight was noted. The experiment was repeated and the mean time taken for three such dimensions was calculated. The result was recorded. The spreadability is calculated by using formula S=M x L/T

Where, S= spreadability

L= length of glass slide

M = weight tied to upper slide

T= time

In present experiment M = 20gm and L = 8.5 cm

The data showing the spreadability of different formulations are shown in the table.

pH DETERMINATION

Healthy human skin reportedly has a surface pH that ranges between 5-7 and a pH gradient exists within the skin. The pH of the gel prepared was measured using a pH meter. The pH was evaluated using 5g of gel; the measurement was reported after 24hrs of manufacture of extemporaneous formulation.

***IN -VITRO* ANTIFUNGAL ACTIVITY OF DEVELOPED FORMULATION**

PROCEDURE

1. Select a pure culture plate of one of the organisms to be tested.
2. Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution.
3. Repeat until the turbidity of the saline solution visually matches that of the standard turbidity.
4. Take a sterile swab and dip it into the broth culture of the organism.
5. Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab.
6. Take a sterile Potato Dextrose Agar (PDA) plate and a nutrient agar (NA) plate.
7. Use the swab with the test organism to streak a NA plate and PDA plate for a lawn of growth.
8. After the streaking is complete, allow the plate to dry for 5 minutes.
9. Antibiotic discs can be placed on the surface of the agar using sterilized forceps.
10. Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loops.
11. Carefully invert the inoculated plates and incubate for 24 hours at 37° C for Bacteria and 5-6 days at 25^{0}C for Fungus.

12. After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.
13. Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone.
14. Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested antibiotic.

RESULT

PROCUREMENT AND IDENTIFICATION OF PLANT MATERIAL

Leaves of *Argemone Mexicana* and *Argyreia Nervosa Were* collected in the month of May from the local and some nearby villages of Amravati district, Maharashtra. The plant species of proposed study was identified and authenticated by Dr. P.W Deotare, Head of the botany department ShriShivaji Science College,Amravati, with reference no SC/BOT/108/2016.

PHARMACOGNOSTIC EVALUATION

MORPHOLOGY

ARGEMONE MEXICANA is an annual herb, growing up to 150 cm with a slightly branched tap root. Its stem is branched and usually extremely prickly. It exudes a yellow juice when cut. It has showy yellow flowers. Leaves are thistle-like and alternate, without leaf stalks (petioles), toothed (serrate) and the margins are spiny. The grey-white veins stand out against the bluish-green upper leaf surface. The stem is oblong in cross-section. Flowers are at the tips of the branches (are terminal) and solitary, yellow and of 2.5-5 cm diameter. Fruit is a prickly oblong or egg-shaped (ovoid) capsule. Seeds are very numerous, nearly spherical, covered in a fine network of veins, brownish black and about 1 mm in diameter.

MORPHOLOGICAL CHARACTERISTICS OF *ARGEMONE MEXICANA*

Sr no	Features	Observations
1	Appearance	Long and Spiny
2	Shape	Cylindrical
3	Length	145-150cm long (2 to 2.5cm)
4	Colour	Gray Brown
5	Surface	Having spine on Upper and Lower Surface
6	Fractured Surface	Rough

Table 2 MORPHOLOGICAL CHARACTERISTICS OF *ARGEMONE MEXICANA*

Fig 3: *Argemone Mexicana* Leafs

Fig 4*: Armona Maxicana* Flowers

Fig 5: *A.Mexicana* Seeds

Fig 6: *A.Maxicana* Fruit

MORPHOLOGY OF *ARGYREIA NERVOSA*

Leaves ovate or cordate, up to 12 in. long, glabrous above, white tomentose beneath, long stalked. **Flowers** in cymes; bracts large, thin, veined, pubescent outside, glabrous inside, calyx white, tomentose outside; corolla funnel shaped, silky pubescent outside, tube somewhat inflated, rose-purple, about 2 in. long. **Root** cylindrical, 1 to 1.5 cm thick; brown, smooth, round wood is scant, flexible, and smooth, latex oozes at cuts. **Seeds** are enclosed in a stone, pale yellow-brown globose apiculate, indehiscent berry 1.2 to 2 cm in diameter containing four erect, curved embryos with corrugated cotyledons or two seeds embedded in a meaty pulp.

Fig 7: Argyreia Nervosa Leaves

Fig 8: Argyreia Nervosa Flowers

Fig 9: Argyreia Nervosa Roots

Fig 10: Argyreia Nervosa seeds

MORPHOLOGICAL CHARACTERISTICS OF *ARGYREIA NERVOSA*

Sr no	Features	Observations
1	Appearance	Silky and glabrous
2	Length	7-15cm
3	Colour	Green
4	Shape	Cordate
5	Length	Petioled
6	Surface	Glabrous

TABLE 3. MORPHOLOGICAL CHARACTERISTICS OF *ARGEMONE MEXICANA* EXTRACTION

EXTRACTION OF *ARGEMONE MEXICANA:* The yield of the extract was 14.34% w/w for petroleum ether, 10% w/w for ethanol, 18.44% w/w for water with respect to dried powder.

Extraction of *Argyreia nervosa:* The yield obtained was found to be 13.23% w/w for petroleum ether, 12% w/w for ethanol, 14% w/w for water.

PHYTOCHEMICAL SCREENING:

Sr.no	Sr.test	Observation	A,maxicana	A,Nervosa
1	Carbohydrates			
	Molisch's test (general test	Violett ring at a junction of two liquids shows the presence of carbohydrate	Negative	Positive
	Tolluen's Phloroglucinol Test	Yellow to Red Colour shows presence of galactose	Positive	Positive
	Iodine test	Blue colour appears which disappears on Boiling and reappears on cooling which shows presence of non reducing polysaccharides (starch)	Positive	Positive
	Fehling's test Reducing Sugar	First Yellow then Red ppt shows presence of reducing sugar	Positive	Negative
2	Proteins			
	Biuret test (general test)	Violet or Pink Colour shows presence of Proteins	Positive	Negative
	Millon's test	White ppt turns Brick red shows presence of Proteins	Positive	Positive
3	Fats and Oils			
	Filter paper test	Permanently Stained with Oil	Positive	Positive
4	Steroids			
	Libermans Buchard's Reaction	First red then blue and finally green colour shows presence of steroids	Positive	Negative
	Salkowski Test	Blue colour appears	Negative	Positive
5	Glycosides			
	Legals Test	Pint to Red colour shows presence of cardenolides	Positive	Positive
	Browntager test	Pinkish red colour to ammonia layer shows presence of Glycoside	Positive	Negative
6	Flavonoids			
	Shinoda Test	Pink colour shows presence of flavonoids	Positive	Positive
	Pews test	Formation of Purple colour or cherry colour indicates the presence of flavonoids	Positive	Negative
7	Alkaloids			
	Dragendorff's Reagent	Orange Brown ppt shows the presence of Alkaloids	Positive	Positive
	Mayer's Reagent	Cream Colour shows presence of Alkaloids	Negative	Positive
8	Tannins and Phenolic compounds			
	Lead acetate test	White ppt shows presence of tannins and phenolic compounds	Positive	Positive
	Bromine water test	Decolourisation of Bromine Water	Positive	Positive

TABLE 4 PHYTOCHEMICAL SCREENING OF PHYTOCONSTITUENTS

The active constituents present in plant extract such as alkaloids, flavonoids, tannins and glycosides present in plants showed its antifungal activity.

***IN -VITRO* ANTIFUNGAL ACTIVITY OF EXTRACTS**

INCORPORATION OF EXTRACTS FOR DEVELOPMENT OF FORMULATION AT DIFFERENT PPM:

Sample no.	Result	Method of analysis
A1 (Extracted with Ethanol)	*Candida Albicans* does not show zone of inhibition at 5000ppm,3000ppm,1000ppm,500 ppm and 25ppm conc. of given compound Compound dissolve in ethanol does not show zone of inhibition.	By paper disc method
A2 (Extracted with Pet Ether)	*Candida Albicans* show a zone of inhibition of 1mm at 5000ppm conc. Where it does not show a zone of inhibition at 3000ppm, 1000ppm, 500ppm and 25ppm conc. of given compound. Compound dissolve in Pet Ether does not show a zone of inhibition.	By paper disc method
A3 (Extracted with Water)	*Candida Albicans* show a zone of inhibition of 3mm at 5000ppm and 2mm at 3000ppm. Where it does not show a zone of inhibition at 1000ppm,500ppm and 25ppm conc. of given compound. Compound dissolve in Water does not shoes zone of inhibition	By paper disc method
B1 (Extracted with Ethanol)	*Candida Albicans* does not show zone of inhibition at 5000ppm,3000ppm,1000ppm,500 ppm and 25ppm conc. of given compound Compound dissolve in ethanol does not show zone of inhibition.	By paper disc method
B2 (Extracted with Pet Ether)	*Candida Albicans* show a zone of inhibition of 1 mm at 3000ppm conc. Where it does not show zone of inhibition at 5000ppm,1000ppm,500ppm and 25ppm conc. of given compound Compound dissolve in Pet Ether does not show zone of inhibition.	By paper disc method
B3 (Extracted with Water)	*Candida Albicans* show a zone of inhibition of 3mm at 3000ppm conc.Where it does not show zone of inhibition at 5000ppm,1000ppm,500ppm and 25ppm conc. of given compound Compound dissolve in Water does not shows zone of inhibition.	By paper disc method

TABLE 5 INCORPORATION OF EXTRACTS FOR DEVELOPMENT OF FORMULATION AT DIFFERENT

PPM (WHERE A1, A2, A3 WERE EXTRACTS OF PLANT *ARGYREIANRVOSA*AND B1, B2, B3 WERE EXTRACTS OF PLANT *ARGEMONE MEXICANA)*

Figure 11 (A2 shows a zone of inhibition of 1mm at 5000ppm).

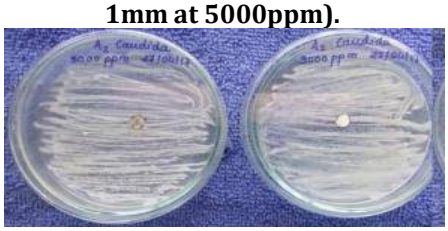


Figure 12 (A3 shows a zone of inhibition of 3mm at 5000 ppm and 2mm at 3000ppm)

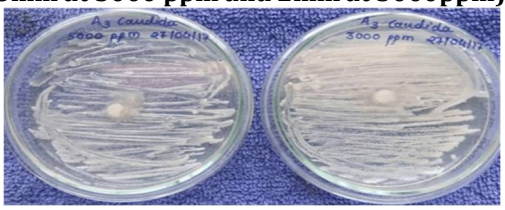


Figure 13 (B2 shows zone of inhibition of 2 mm at 3000ppm)

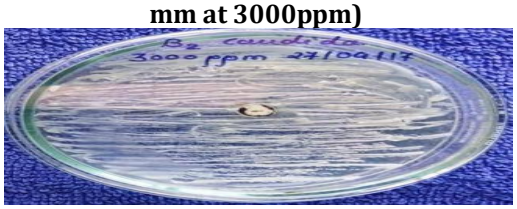


Figure 14 (A3 shows a zone of inhibition of 3mm at 5000 ppm and 2mm at 3000ppm)

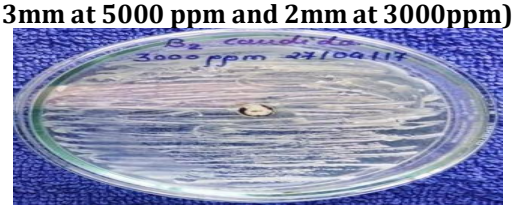
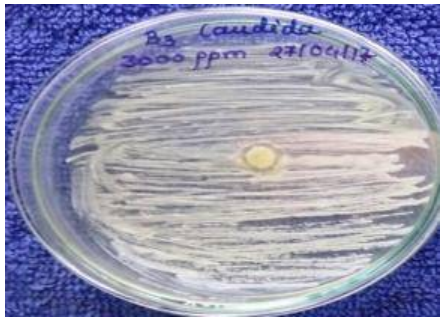


Figure 15 (B3 shows a zone of inhibition of 3mm at 3000ppm)



It was found that the plant extracts of *Argyreia nervosa* and *Argemone mexicana*in petroleum ether and water showed more potent antifungal activity.

FORMULATION DEVELOPMENT

As the extracts were prepared in different solvents with the help of Soxhlet extraction the selected extracts of water and pet ether were selected for the preparation of formulation F1 and F2 as per the conc/ppm of the anti-fungal activity shown at different ppm of 3000ppm and 5000ppm.

FORMULATION BATCHES

	F I	**F II**
Ingredients	**Quantity Taken**	**Quantity Taken**
Argeria nervosa extract in water	0.4	0.3
Argemona mexicana extract in water	0.3	0.2
Aloe Vera gel Extract	2	2%
Cetostearyl alcohol	1%	-
Methyl Paraben	0.5	0.2
Propylparaben	0.5	0.1
Disodium EDTA	Q.S	-
Propylene Glycol	2%	2%
Triethanolamine	Q.S	Q.S
Water	100ml	100ml

TABLE 6 FORMULATION BATCHES

PHARMACEUTICAL EVALUATION OF ANTI-FUNGAL GEL

Sr No.	**Viscosity**	**Thermal Stability**	**Spreadability**	**PH**	**Extrudability**	**Appearance**	**Colour**	**Homogenecity**
F1	16540	No separation	6.85	6.2	72% Fair	Smooth	Dark green	Homogenous
F2	20230	No separation	7.14	6.8	82% Fair	Smooth	Dark green	Homogenous

TABLE 7 EVALUATION OF ANTIFUNGAL GEL

***IN-VITRO* ANTIFUNGAL ACTIVITY OF GEL FORMULATION**

The antifungal activity of samples against *Candida Albicans* using the paper disc method showed that extracts had significant activity against fungal strain,while combined samples also showed moderate activity against *Candida Albicans*. Antifungal activity of formulations prepared from water and pet ether extract of plants showing its activity at different concentrations of 3000ppm and 5000ppm against Fluconazole as standard.

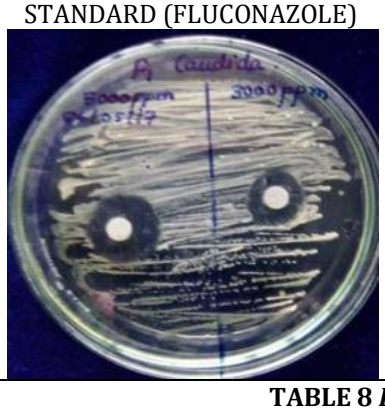
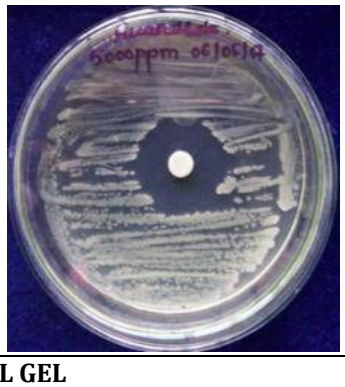
Sample no.	**Result**	**Method of analysis**
F1 Combination of A2+B2 Extract	*Candida Albicans* show a zone of inhibition of 6mm at 5000ppm conc, 4mm at 3000ppm conc Compound dissolve in Pet Ether does not show a zone of inhibition.	By paper Disc method
F2 Combination of A3+B3	*Candida Albicans* show a zone of inhibition of 3mm at 3000ppm conc.Compound dissolve in water does not show zone of inhibition.	By paper Disc method
STANDARD (FLUCONAZOLE) 	*C.ALBICANS* SHOWS A ZONE OF INHIBITION OF 10MM AT CONC. OF 5000PPM OF DISC OF STANDARD FLUCONAZOLE. 	BY PAPER DISC METHOD 

TABLE 8 *IN-VITRO* EVALUATION OF ANTIFUNGAL GEL

The antifungal activity of *Argyreia Nervosa* and *Argemone Mexicana* was noted as 6mm zone at 5000ppm conc and 4mm zone at 3000ppm conc for F1 and no zone of inhibition at 5000ppm concentration and 3mm zone at 3000ppm concentration against the standard fluconazole STD which shows the zone of inhibition of 10mm at 5000ppm concentration. The development of formulation using extracts incorporated in *Aloe vera* gel and other preservatives was prepared successfully and its antifungal activity was again tested against the standard (Flucanazole at 3000 and 5000ppm).

The study includes phytochemical investigation of plant extracts of *Argyreia nervosa* and *Argemone mexicana* and development of extraction process and isolation process for further formulation development. The leaves of *Argyreia nervosa* and *Argemone Mexicana* were collected from the campus of Government College of Pharmacy, Amravati, and other rural regions of Amravati [1-6]. Leaves were dried under shade and subjected for size reduction to get coarse powder and then to form uniform powder. Then powder was subjected to standardization with different parameters as per literature. After that plants are extracted with respect to different solvents, after extraction phyto-chemical investigation is carried out. These different extracts were evaluated for more potent in vitro antifungal study in different solvent used for development of two different gel formulations using *Aloe vera* gel as natural gel base and the incorporation of extracts were done in the prepared gel base. Formulation and development of gels, its evaluation for various parameters were carried out. Herbal formulation also passes thermal stability. After formulation development by using specific extract and its evaluation, the *in vitro* antifungal study was again carried out, the formulation containing extract of *Argyreia nervosa* and *Argemone mexicana* in pet ether showed moderate antifungal effect against *Candida albicans* [7-13].

CONCLUSION

Our findings suggest that alkaloids, flavonoids, and glycosides present in fractions may be potential for enhancing the antifungal activity.

NEED AND SCOPE

Fungal infections of the skin are one of the often faced with dermatological diseases worldwide. Topical therapy is an attractive choice for the treatment of cutaneous infections due to its advantages such as targeting drugs to the site of infection and reducing the risk of systemic side effects. Currently, antifungal drugs are generally used as conventional cream and gel preparations in topical treatment. The efficiency of that treatment depends on the penetration of drugs through the target layers of the skin at the effective concentrations. However, stratum corneum, the outermost layer of the skin, is an effective barrier for penetration of drugs into deeper layers of the skin. The physicochemical characteristics of drug molecules and the types of the formulations are effective factors in topical drug delivery. Therefore, a number of formulation strategies have been investigated for delivering antifungal compounds through targeted sites of the skin. The focus is on the new alternative formulation approaches to improve skin penetration of antifungal natural pharmacognostic drugs.

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