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Introduction

"***All knowledge that the world has ever received comes from the mind; the infinite library of the universe is in our own mind***"

-Swami Vivekananda

Nature provides all the resources in terms of environment, food, shelter, water, *etc*. Among these, plants are essential gifted sources by nature, and it satisfies all needs of living organisms. Moreover, the plants contain numerous medicinal values, and the scientific valediction of the medicinal plants is essential for the research communities by way of a green revolution. Therefore, every research should have an application for a healthy society. In this way, this study focuses on utilizing the traditional medicinal samples in pharmacological and cosmetic applications. The importance of this study is discussed systematically in this section.

1.1 Importance of unwanted hair removal

Removal of unwanted hair from the human body is a part of the human being's living style. It is indispensable for attractiveness, personal grooming **(Tatlidede *et al*., 2018)**, sexuality, and aesthetic reasons **(Andrea *et al*., 2014)**. Most women are showing very much interest in removing unwanted hair. Nowadays, men are also concerned about this problem. Axillary hair can influence to develop the underarm odour in men **(Lanzalaco *et al*., 2015)**.

Various methods (shaving, epilation, depilation, laser, electrolysis, *etc*.) remove unwanted hair. The hair removal options vary by their efficacy, cost, and discomfort. Most of the studies are not clinically proven. The permanent hair removal is electrolysis and laser technique. Electrolysis lacks standardization and laser needs a more regulated system and this is to be yet proven. Shaving, depilation, and epilation are standard and less expensive methods, but these only provide temporary results **(Donald and Virginia, 2002)**. Commonly minor complications occur during hair removal, and this will negligible by discussing with Gynecologically **(Andrea *et al*., 2014)**.

Hair removal is also essential during surgery. Approximately 2 million surgical patients are affected by surgical site infection in the US, and more than 80,000 patients have died. Shaving is a traditional method for removing hair during surgery **(Mohammed and Hamedan, 2016)**. It is a simple, easy method, and it has some disadvantages like difficulties in shave in folds, perineal, axillae, scrotal parts of the body, and it is very painful in the sensitive areas. Infection is also possible in this method due to improper sterilization of shaving tools and the transaction of microorganisms from patients to patients **(Prigot *et al*., 1962)**. Laser treatment requires training to handle lasers and regular maintenance for removing hair during surgery **(Tejero *et al*., 2015)**.

Around worldwide, women (10%) are affected by the severe condition of Hirsutism, leading to psychological suffering. The efficacy of the topical capislow and placebo applied after the laser treatment are studied. The combined topical capislow with laser is a protected and successful synergistic technique to acquire quicker outcomes **(Hisham *et al*., 2018)**. **Lim and Lanigan, 2006** reviewed the later effects of laser treatment. The side effects are low, and it is very uncommonly permanent for all skin types. It may create pain, oedema blistering, cold urticaria, crusting folliculitis, perifollicular erythema, thrombophlebitis, purpura, hyperpigmentation, hypopigmentation, reticulate erythema, erosions, scarring, postoperative expulsion of harmed hair shafts, hypertrichosis, folliculitis, and incomprehensible hair development in untreated areas. The permanent effects are Atrophy and Scarring. All these effects strongly depend on skin type.

Creams and lotions are commonly available forms of chemical depilatories. Calcium hydroxide and potassium thioglycolate are commonly used chemicals in depilatory creams and are essential to dissolve and weaken the hair keratin protein. Toxic metals in depilatory creams cause skin redness, inflammation, irritation, skin blackening, and it also causes hormonal cancer, sexual problems, and rashes on sensitive skin **(Qurat and Iftikhar, 2013)**. Thioglycolates present in the depilatory creams effectively enhance transdermal drug delivery and increase the penetration of toxic chemicals to the *stratum corneum* of the skin **(Lee *et al*., 2008)**. The need for depilation is not only for personal development but also for the leather industry. The depilation process is used for fibre opening and unhairing. The pollution occurred in the leather industries by sodium sulphide, and Ca_2OH is replaced by Ionic liquid **(Jaya Prakash *et al*., 2017)**.

Depilatory cream shows minor complications compared with shaving on the surgical site, and it significantly reduces the infection by 4.88% (12.16% surgical site infection using razor) in the surgical site. No erythema and 1.22% rashes were observed using depilatory cream (12.16% erythema and 4.05% rashes observed using razor) **(Chetty *et al*., 2017)**.

Skin injuries (4%) and infection in the wound area (3%) is reduced while using depilatory creams. The use of razors resulted in 29% of skin injuries and 13% wound infection. Complete hair removal (91%) was observed using depilatory cream and 62% using a razor **(Suvera *et al*., 2013)**. **Adisa *et al*., 2011** reported that 88.6% of hair was removed entirely (61.6% for razors), 3.8% for skin injuries (27.9% for razors), and 2.5% for wound infection (12.8% using razor) caused using a depilatory cream. Using depilation cream does not show any itching and discomfort during the hair regrowth **(Powis *et al*., 1976)**. Hair removal using creams is a painless and fast method (3-15 min, which depends on the cream and type of the hair used), and no development of cuts and nicks occur while using depilatory creams. It also shows some disadvantages of a strong unpleasant smell, burning the skin by chemicals left on the skin for a long time **(Jyothi *et al*., 2012)**.

Due to health and environmental concerns, the present study aimed to prepare non-toxic depilatory creams via utilizing traditional based depilatory agents.

1.2 Plant-based research on medicinal applications

Plants are an important renewable source for satisfying the demands of the people for their day-to-day life. Before development of science and technology, our ancestors had been more knowledgeable about handling many plants to cure numerous diseases; it is also evidenced in our ancient literature. In Egyptian dating (before 1500 BC), peoples were more knowledgeable about the plants for food, pharmacological effect, and quality **(Sekar *et al*., 2013)**. Each part (flower, leaves, fruits, bark, root, rhizome, *etc*.) of the plant possess unique medicinal properties **(Bamola *et al*., 2018)**.

In developing countries, 80% of the total population depends on plant sources for their daily needs. World Health Organization estimation reveals the same percentage (80%) of the total population to depend on plant-based drugs in future **(Hishe *et al*., 2016)**. Among the 7.5 billion total population, 4.5 billion people use traditional medicine **(Kamala *et al*., 2018)**. During the three decades, ~50% of herbal drugs from natural products are approved. The plants contain several pharmacological activities (antimicrobial, antioxidant, antiviral, anticancer, antiparasitic, *etc*.), and plant-based medicines have lesser side effects and readily available **(Shakya, 2016).**

Medicinal plants possess many important phytoconstituents (alkaloids, phenols, carbohydrate, flavonoids, sterols, *etc*.) responsible for their medicinal properties. Quinine (Alkaloids) is a commercially available antibiotic for malaria, isolated from Cinchona and other alkaloids such as caffeine, morphine, and nicotine used for analgesic activity. Aspirin is isolated from the bark of the willow. Luteolin and catechins are flavonoids that show good antioxidant activity than other nutrients. Diterpenoid is a terpenoid used as an anticancer drug **(Saxena *et al*., 2013)**. Most of the saponins are act as antimicrobial agents for insect attacks **(Lacaille and Wagner, 2000)**. The naturally derived compounds strongly interfere with and prevent all types of cancer **(Altemimi *et al*., 2017)**. The usage of antibiotics with higher inhibition efficiency is emphatically going with the expense of the treatment. This will be avoided by utilizing plant-based antibiotics **(Gulbagca *et al*., 2019)**.

Due to the advantages of medicinal plants in terms of easy availability, non -toxic nature and numerous chemical constituents, plants are chosen in the present study.

1.2.1 *Cyperus rotundus* Linn

Cyperus rotundus is a type of perennial weed belonging to the Cyperaceae family. It is commonly known as purple nutgrass, and it is considered traditional herbal medicine in various countries (India, China, Iraq, *etc*.). It consists of a black colour tuber with a pleasant odour. Due to this aroma of the tuber, it is used in many perfumes and soaps. This aroma is because of secondary metabolites present in the tuber portion. The essential oil in the tuber portion is used in cosmetics and for various medicinal applications.

Cyperus rotundus shows antibacterial activity against *Staphylococcus aureus, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and antifungal action against *Candida albicans* and *Aspergillus niger* **(Ali Abdella and Hajar, 2014)**. It exhibits antidepressant effect **(Hao Gui *et al*., 2017)**, mosquito repellent **(Singh *et al*., 2009)** and antioxidant activity **(Pal and Dutta, 2006; Jeyasheela *et al*., 2011; Lydia and Sundarsanam, 2012; Ali Abdella and Hajar, 2014; Ismahen *et al*., 2014; Amjed *et al*., 2018)**. The phenols and flavonoids present in the tuber portion are responsible for the antioxidant property **(Krishna and Renu, 2013)**.

It shows allelopathic activity **(Ilham *et al*., 2018)**, anticonvulsant activity **(Shivakumar *et al*., 2009; Mayur *et al*., 2011)**, antiangiogenic activity **(Amjed *et al*.,**

2018), antidiabetic activity **(Raut and Gaikwad, 2006; Pradeep *et al*., 2019)**, decrease joint pain by using cream formulation **(Rahim *et al*., 2016)**, anti-inflammatory **(Ahmad *et al*., 2014; Nidugala *et al*., 2015)**, inhibit cariogenic properties of *Streptococcus mutans* **(Yu Hyeon *et al*., 2007)**, antimicrobial **(Wangila, 2017; Vijender *et al*., 2018)**, antiulcer activity **(Mohammad *et al*., 2012; Ahmad *et al*., 2014)**, ovicidal and larvicidal activity **(Vivek and Bhat, 2008)**. The tuber effectively shows inhibition against diarrhoea infectors **(Poonam *et al*., 2011)** and it also shows wound healing activity **(Puratchikody *et al*., 2006)**.

Cyperus rotundus cures many diseases with a combination of other plants. The tuber is mixed with *Tinospora cordifolia* and dried ginger as medicine for malarial fever. *Cyperus rotundus* tuber with *Fumaria indica* (leaves), black pepper, *Swertia chirayita*, and ginger is used for typhoid fever. Mixing with ginger and honey is used against dysentery and gastric problems **(Vijender *et al*., 2018)**. It is used for stomach aches and is called an analgesic plant and folk medicine in Turkey **(Ilham *et al*., 2018)**. It is also a constituent of Kabasura neer recommended in COVID.

Cyperus rotundus oil effectively decreases hair growth. *Cyperus rotundus* essential oil shows androgenic action on androgenic hair; this might be because of the flavonoids present in the oil. Axillary hair was effectively removed by *Cyperus rotundus* essential oil without disturbing the testosterone of serum. The development of sexual hair is entirely reliant on the nearness of androgen (Androgenic hair) **(El-kaream, 2012)**. *Cyperus rotundus* essential oil is used along with the Alexandrite laser for diminishing the development of both dark and white hair with no adverse reactions **(Ghada, 2014)**.

1.2.2 Pumice stone

Pumice is a readily available lightweight stone obtained from volcanic eruptions. All over the world, 50 countries produce different pumice stone products **(Sivalinga *et al*., 2013)**. It is available in white colour with even texture. It is a lightweight aggregate used in Roman structures. It has low specific gravity, is highly porous and has higher water absorption capacity **(Bhavana and Rambabu, 2017)**. Pumice stone produced by the forceful release of rock by higher temperature and pressure in rock has an approximate density of 500-600 Kg/m^3 **(Manzoor *et al*., 2018)**.

Pumice stone is a water-floating stone due to gas release from the molten lava during solidification **(Shambharkar *et al*., 2018)**. It has a microporous (85%) structure with a high specific surface area **(Mohammad *et al*., 2013; Ulker and Sarioglu, 2014)** and it is vital for avoiding calcinations **(Derakhshan *et al*., 2013)**. A pumice stone can also form by mixing lava with hot water, followed by rapid cooling and depressurization. The pores are caused by decreasing the solubility of water and dissolving CO_2 during depressurization. It possesses a high-water absorption capacity (20-30%), mainly made of SiO_2 and Al_2O_3 **(Ersoy *et al*., 2010)**.

During the world war, the pumice stone served an essential role in constructing ships and barges by military engineers due to its lightweight properties **(Payal and Siba, 2017)**. It has high mechanical strength, high melting point, fire-resistant, excellent insulating, and soundproofing properties **(Muralitharan and Ramasamy, 2015)**. Pumice stone possesses numerous medicinal values, and its pharmaceutical application started over 2000 years ago. In the mid-eighteenth century of China, it was used as an ingredient in tea with a combination of other ingredients to treat dry coughs, gall bladder cancer, and anxiety disorders. In the early days, a pinch of pumice added during boiling wine reduced the boiling point. It is used to clean the teeth and body. In ophthalmic making, the pumice powder was used for ulcerations of the eyes (act as a lenitive detergent), cicatrizing agents for wound care, and as the active ingredient in eye ointments. Pumice stone is used to remove body hair due to its abrasive behaviour **(Christopher, 2012)**.

Micro fined grains of pumice stone give a magnificent exfoliating medium to evacuate dead and harsh skin **(Estanqueiro *et al*., 2012)**. It acts as an anti-irritant agent due to the presence of silicon dioxide, and it was increasing the emulsions **(Estanqueiro *et al*., 2014)**. Milia cysts in the face will completely disappear by sprinkling the fine powder of pumice stone **(Herbert, 1957)**. Mainly the pumice stone is used by women for cleaning their feet (for removing calluses). It is used for removing hyperkeratotic tissue in combination with keratolytic agents **(Howard and Boston, 1980)**.

It has other applications also. Due to its lightweight properties, it used as a block of concrete in lintels, sunshades, partition walls and other building structures **(Sivalinga *et al*., 2013; Muralitharan and Ramasamy, 2015; Fauzi *et al*., 2016; Tanveer *et al*., 2016; Caiza *et al*., 2018; Manzoor *et al*., 2018; Meyyappan *et al*., 2019; Sangeetha *et al*., 2020)**. Composite of the enzyme and pumice stone helps to wash the garments **(Sarkar *et al*., 2014a)**, removing Tetracyline (TC) antibiotics from aqueous solution **(Ulker and Sarioglu, 2014)**, removal of copper from water and remove heavy metals such as nickel, zinc, cadmium, *etc*., **(Bilgehan and Mesut, 2016)**. Ni-pumice composite is an alternative coating material instead of Ni-SiC which helps to coat the trochoids of Wankel engines **(Aruna *et al*., 2014)**.

Pumice stone is used to remove Pentachlorophenol (toxic phenolic compound) and contaminants from water and wastewater **(Ali Reza *et al*., 2018)**. Pumice stone coated TiO_2 helps to deactivate bacteria (*Escherichia coli* exist in river water) and degradation of organic dyes (acid orange-7, resorcinol 4, 6-dinitro-o-cresol, 4-nitrotoluene-2-sulphonic acid and isoproturan) **(Subrahmanyam *et al*., 2008)**. The pumice stone is involved in absorption of Methylene Blue dye **(Mohammad *et al*., 2013)**, Azo dye **(Veliyev *et al*., 2006)** and Acid Red 14 **(Samarghandi *et al*., 2012)**. Using a pumice stone, the adsorption of water hardening cations of Ca^{+2}, Mg^{+2} **(Sepehr *et al*., 2013)**, Acid Red 14 and Acid Red 18 **(Reza *et al*., 2012)** is observed. Removal of cadmium from wastewater is effective with iron nanoparticle-coated pumice **(Shokoohi *et al*., 2016)**.

1.2.3 *Hemigraphis alternata* (Burm.f.)

Hemigraphis alternata is one of the important readily available medicinal and perennial plants belonging to the family of Acanthaceae. It grows both indoor and outdoor with attractive and vivid foliage **(Nair and Nair, 2018)**. In Kerala, it is known as Murikootti and Muriyan pacha, the name indicates the plant's application (Muri means to cut or wound) and used as folk medicine. The leaves are red-purple-green colour on the surface and a darker purple colour under the leaves. The plant is used as drugs, dyes, and food supplements **(Reshma *et al*., 2018)**. Paste from the leaves promotes wound healing **(Subramoniam *et al*., 2001; Silja *et al*., 2008; Sheu *et al*., 2012)** and is also used to treat anaemia **(Devi Priya, 2013)**. It shows wound healing activity **(Joo, 2016)** on fibroblasts and endothelial cells **(Boby and Prabha, 2011)**. It is used for antidiabetic treatment **(Gayathri *et al*., 2012)**.

The aqueous extract of *Hemigraphis alternata* leaves shows inhibitory and mitodepressive effects on the *Allium cepa* cell division than control. This effect may use to identify uncontrolled cell division of tumour. In every stage of Mitosis, Chromosome aberrations are noted **(Hilal, 2019)**. Synthesized silver nanoparticles using *Hemigraphis alternata* showed significant anti-inflammatory activity **(Thomas and Mathew, 2019)**. *Hemigraphis alternata* incorporated chitosan scaffold indicates antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. It shows cell viability (90%) on human dermal fibroblast cells **(Annapoorna *et al*., 2013)**. It shows anti-inflammatory and anti-nociceptive activity **(Rahman *et al*., 2019)**.

The leaves show removal efficiency on five volatile indoor pollutants (benzene, toluene, octane, trichloroethylene and α-pinene) **(Dong *et al*., 2009)**. It exhibits antibacterial activity against *Staphylococcus aureus* (38 mm) and *Proteus mirabilis* (34 mm), antifungal activity against *Penicillium notatum* and *Candida albicans* **(Agneeswari and Jansi, 2019)**. It shows growth inhibition activity against *Bacillus* species., *Staphylococcus aureus*, *Enterococcus sp.*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Moraxella* species, and *Serratia plymuthica*. The whole plant is used to stop external bleeding **(Neenu *et al*., 2018)**.

Considering the traditional behaviour of Cyperus rotundus and pumice stone in unwanted hair removal applications and their various biological activities, this study focuses on preparing non-toxic free depilatory creams and assessing their depilatory action. In addition, due to its medicinal value, Hemigraphis alternata is also utilized in this study.

1.3 Importance of biological activity studies

1.3.1 Skin cancer activity

Cancer is one of the non-communicable diseases responsible for the second leading death causes all over the world. Annually, in India, ~5,00,000 people die due to cancer and the disease is expected to rise five-fold by 2025 **(The Times of India, 2014)**. All living organisms consist of several organs with multiple functional activities. Skin is the most needed organ to sustain the life of all types of living organisms. It used to shield from the contact of undesirable outside variables. In addition, it helps detect oxygen absorption, keep up the internal heat level, forestalling water loss, and create vitamin D **(Tabassum and Hamdani, 2014)**. The *stratum corneum* is a central part of the skin, which plays a vital role in protecting nerves, muscles, blood vessels, and infectors (bacteria, fungi, and virus) **(Yagi and Yonei, 2018)**. Therefore, we should be aware of maintaining the skin from various diseases (cancer, infection, wound, *etc*.).

Today's abnormal pollution and environmental changes are mostly taking responsibility for creating many diseases. Skin cancer is one of the worst cancers among living organisms because it immediately shows outside. Malignant and non-malignant melanoma are the main types of skin cancer. Squamous Cell Carcinoma (SCC) and Basal Cell Carcinoma (BCC) come under non-malignant melanoma skin cancer. This post-developed cancer is hazardous and difficult to cure **(Silpa and Chidvila, 2013)**. SCC and BCC represent 80 and 16% skin cancer, respectively, remaining (4%) due to melanoma **(Greenlee *et al*., 2000)**.

Over the past 20 years, the effect of BCC (35%) and SCC (13%) increased **(Pinton *et al*., 2015)** due to the penetration of UV radiation (95%), carcinogenic chemicals, ionizing radiation, and release of hazardous from vehicles smoke (hydrocarbon) **(Chummun and McLean, 2017)**. UV (A and B) radiation causes immunosuppression and DNA damage by producing reactive oxygen in the skin **(Andrew *et al*., 2013)**. Many treatments are available to cure cancer (surgery, therapy, creams, *etc*.) but these are having some side effects (loss of hair, weight, pain in muscle, fatigue, nausea, and diarrhoea) **(Baozhong and He, 2010; Berking *et al*., 2014)**. The creams possess many side effects. Imiquimod is an immunotherapy cream that showed fever, rashes, itching, joints aches and headache. 5-fluorouracil is a chemotherapy cream that showed blister, peel, and red crack on the skin, which creates discomfort **(Mothoneos, 2020)**.

Cancer treatment is expensive because of the requirements of a lot of medicines and treatments. The cost of diagnosing skin cancer (1,50,000 incident cases) is $536 million in Australia **(Christopher *et al*., 2015)**. Due to the advantages of cost-effectiveness, non-toxic and availability, the plant-based researches enhanced among the researchers. Many antibiotics (60%) are available for cancer treatment from medicinal plants **(Wang *et al*., 2019)**. Some medicinal plants also have the potential for skin cancer treatment. *Camellia sinensis* (tea extract) **(Katiyar, 2011)** and *Tilia amurensis* were indicating toxicity against skin melanoma cells **(Kim *et al.*, 2012)**. Myricetin, *Cissus quadrangularis* Linn **(Bhujade *et al*., 2013; Sun Wei *et al*., 2018),** *Vanilla planifolia* **(Vijaybabu and Punnagai, 2019)**, oil from jasmine **(Manjunath and Mahurkar, 2019)**, *Acanthus ebracteate* **(Khamwut *et al*., 2019)**, *Etlingera elatior*, *Rosa damascene*, torch ginger, and *Rafflesia kerrii* **(Thuncharoen *et al*., 2013)**, *Caralluma R.br.* and *Boucerosia indica* **(Vajha *et al*., 2014)** showed anticancer activity against skin cancer cell lines.

1.3.2 Antimicrobial activity

Many internal and external factors are responsible for hospitalizing patients **(Bereket *et al*., 2012)**. Most child deaths are caused in low and middle-income countries due to bacterial infection **(Liu Li *et al*., 2016)**. In the US, annually 1% of infection and ~12000 deaths occur due to *Staphylococcus aureus* **(Noskin *et al*., 2005)**. Reports on Centers for Disease Control and Prevention, NCEZID and DHQP, 2019 states that in the US every year, there are >2.8 million infections and 35,000 deaths caused **(https://www.cdc.gov/drugresistance/biggest-threats.html)**. In the US, every year ~73,000 people are affected **(Rangel *et al*., 2005)** and ten million deaths caused by

Escherichia coli. In 2050, $100 trillion costs are expected on antimicrobial resistance **(Simpkin *et al*., 2017)**. Infection due to *Pseudomonas* mainly occurs among patients having blood group A and *Klebsiella*, and *Staphylococci* infection occurs for blood group O **(Abdulhamid *et al*., 2013)**. The fungus is primarily affecting the nails and skin in humans **(Singh *et al*., 2014)**.

According to the National Institute of Health, 65% of microbial infections are associated with biofilm formation. Microbes initially attach to the surface of living and non-living surfaces. Then it forms microcolony, 3D structure, and finally attain maturation with detachment. Bacteria are contacting one another, resulting in quorum sensing during biofilm formation **(Jamal *et al*., 2018)**. By increasing the environmental discharges by a human, the infections also increase during bacteria growth. The diseases are caused directly and indirectly by bacteria and infectors to the environment **(Ghosha *et al*., 2006)**.

The plants protect and fight themselves from many infectors. *Cyperus rotundus* (aerial) shows antibacterial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Escherichia coli* **(Jaziri *et al*., 2011)**. *Cyperus rotundus* essential oil from rhizomes shows cell membrane damage and apoptosis against *Staphylococcus aureus* **(Liang *et al*., 2017)**. *Pseudomonas aeruginosa* a gram-negative bacterium responsible for skin, eye, ear, respiratory, bone and cartilage tissue infection is inhibited using ethanolic extract of *Cyperus rotundus* tuber **(Dadook *et al*., 2016)**.

Cyperus rotundus shows antibacterial activity against *Escherichia coli* (Ethanol extract), *Bacillus subtilis* (butanol extract), *Staphylococcus aureus* (methanol extract) and *Streptococcus faecalis* (butanol extract) **(Prasad, 2014)**. The isolated compound (Lup-12, 20 (Lupenyl arabinosyl oleate) from *Cyperus rotundus* ethanolic extract shows bacterial inhibition against *Staphylococcus epidermidis* and antifungal activity against *Aspergillus niger* **(Singh and Surendra, 2015)**. *C. rotundus* ethanolic extract shows excellent antifungal activity against *Staphylococcus aureus*, *Aspergillus niger* and *Escherichia coli* **(Adeniyi *et al*., 2014)**.

Ethanolic extract of *Cyperus rotundus* shows antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* **(Puratchikodi *et al*., 2015)**. It shows antifungal activity against *Botrytis cinerea* (high inhibition), *Fusarium oxysporum f. sp*. radicis lycopersici (low inhibition) and it shows antibacterial activity against *Pseudomonas syringae* **(Ozdemir and Erincik,**

2015). Ethanol extract shows activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. It shows antifungal activity against *Aspergillus niger* and *Candida albicans* **(Kabbashi *et al*., 2015)**. *Bacillus subtilis*, *Bacillus pumilus* and *Candida albicans* (antifungal) are inhibited using volatile oil of *Cyperus rotundus* **(Gupta *et al*., 2016)**.

The critical challenges are, discovering the anticancer and antimicrobial drug with low toxicity to the normal cell and high toxicity to cancer cells. This can be overcome by employing plant-based nanotechnology and where the nanoparticles are used only at the nano or micro level. The biological activity using selected samples is carried out in this study due to the importance of the antimicrobial and anticancer drugs.

1.4 Nanotechnology and nanoparticles

All over the world, nanotechnology creates many opportunities in all types of research with numerous applications (electronics, health, medicine, sensors**,** and biomedical) **(Nikalje, 2015; Khenfouch *et al*., 2016)**. Nanoparticles are biocompatible **(Noruzi, 2015)** with unique physical and chemical properties, photonic, and specific drug delivery **(Dreaden *et al*., 2012)**. The main challenges involved in this mission are to move towards green synthesis and adopting an environmental process. Bio-mediated nanoparticle synthesis is an alternative method to produce metallic nanoparticles. Plants are readily available, non-toxic, inexpensive, and contain more capping agents **(Noruzi, 2015)**. The method consists of simple techniques, low cost, and low cytotoxicity. Nanoparticles will help achieve active uptake, reduce the metal ions in a solution, and form neutral gold atoms **(Klekotko *et al*., 2016)**.

1.4.1 Gold nanoparticles

Gold nanoparticles are not new; it has been utilized by our ancestors before 400 years. It is used for staining the glass and enamels **(Armendariz *et al*., 2004)**. Among the other noble metals (platinum and silvers), gold possesses numerous applications due to its excellent functional properties and a broad spectrum of activity against microorganisms. Gold nanoparticles have a higher surface area compared to gold ions. It is used as a surface coating material for medical devices, and dental resin composite **(Senthilkumar *et al*., 2017)**. Chemical and physical methods are commonly used to synthesize gold nanoparticles, but many researchers show their interest in preparing gold nanoparticles by eco-friendly adopted techniques; this problem is overcome by plant-based synthesis.

Plant-based synthesized gold nanoparticles has valuable application such as drug delivery **(Pandey *et al*., 2012; Devendiran *et al*., 2014; Muhammad *et al*., 2017)**, photocatalytic activity **(Brajesh *et al*., 2015)**, converting borohydride to different isomeric nitrophenols **(Phukan *et al*., 2016)**, catalytic reduction of 4-nitrophenol to 4-aminophenol (**Majumdar *et al*., 2013; Shib and Braja, 2014; Shib *et al*., 2015; Subhajit *et al*., 2015; Kumar *et al*., 2016; Soo *et al*., 2016; Sunkari *et al*., 2017; Mapala and Pattabi, 2017)**, catalytic activity of nitro-organic pollutant (o-nitroaniline) **(Dauthal and Mausumi, 2016)**, oxidation of 1, 8-cineole to 2-oxo-1,8-cineole **(Ashwani and Nrivastava, 2015)**, carbendazim detection in soil samples **(Li and Zhang, 2016)**, antioxidant activity **(Lakshmanan *et al*., 2016; Brajesh *et al*., 2016; Nithya and Jayachitra *et al*., 2016; Madhanraj *et al*., 2017; Saad *et al*., 2017; Francis *et al*., 2018)**, degradation of Methylene Blue **(Kannan and Hyun, 2015; Yulizar *et al*., 2017; Francis *et al*., 2018)** and Rhodamine **(Francis *et al*., 2018)**. It is involved in chemo catalytic activity **(Ghosh *et al*., 2012)**, sensitive colorimetric detection of cysteine **(Bagci *et al*., 2015)**, hepatoprotective activity **(Belliraj *et al*., 2015)**, scavenging activity **(Dauthal and Mausumi, 2013; Nakkala *et al*., 2015; Balasubramani *et al*., 2016)**, colorimetric sensor to detect and estimate the pesticide and methyl parathion **(Barman *et al*., 2013)**.

Anticancer and antimicrobial activity of medicinal plants aided gold nanoparticles is listed here. Prepared gold nanoparticles using *Andrographis paniculate* leaves shows anticancer activity against HeLa and MCF-7 cell lines **(Babu *et al*., 2012)**, *Corallina officinalis* **(Yassin and Mostafa, 2014)** and *Allium cepa* **(Parida *et al*., 2011)** shows cytotoxicity against MCF-7 cell lines. *Shorea tumbuggaia* **(Li Yuan *et al*., 2017)**, *Gymnema sylvestre* **(Nakkala *et al*., 2015)**, *Leucas aspera* **(Prabu *et al*., 2016)** and *Acalypha indica* Linn **(Krishnaraj *et al*., 2014)** has potential activity against SW579, Hep-G2 cells and MDA-MB-231 cell lines respectively. *Rhus chinensis* shows cytotoxic activity against MKN-28, Hep-3B and MG-63 **(Patil and Gun, 2017)**. *Mappia foetida* exhibit anticancer activity against MDA-MB-231, HeLa, SiHa and Hep-G2 **(Yallappa *et al*., 2015)**, *Fagopyrum esculentum* shows cytotoxicity against human IMR-32, MCF-7, and HeLa cancer cell lines **(Babu *et al*., 2011)**.

Naregamia alata aided gold nanoparticles show bacterial inhibition against *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus nidulans*, *Escherichia coli*, *Aspergillus flavus* and *Pseudomonas aeruginosa* **(Francis *et al*., 2018)**. *Delonix elata* leaves show antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris* and *Streptococcus pyogenes* **(Akilandeswari and Sathya, 2017)**. *Prosopis juliflora* shows antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* **(Rao *et al*., 2017)**. *Callistemon citrinus* shows against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* **(Saad *et al*., 2017)**. *Aegle marmelos* shows inhibition against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* **(Arunachalam *et al*., 2013)**. *Turbinaria conoides* exhibit inhibition activity against *Bacillus subtilis*, *Klebsiella planticola* and *Streptococcus pyogenes* **(Kumar *et al*., 2013)**. *Bacopa monnieri* shows antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* **(Mahitha *et al*., 2013)**.

Azima tetracantha Lam aided gold nanoparticles shows antibacterial activity against *Aeromonas liquefaciens*, *Salmonella typhimurium*, *Micrococcus luteus* and *Enterococcus faecalis*. It also exhibits antifungal activity against *Candida albicans*, *Microsporum canis*, *Cryptococcus* species and *Trichophyton rubrum* **(Hariharan *et al*., 2016)**. *Couroupita guianensis* shows against *Bacillus cereus* **(Kirbha and Alagumuthu, 2014)**. *Ficus benghalensis* shows inhibition against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis* **(Francis *et al*., 2014)**. *Costus igneus* exhibit antibacterial activity against *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* **(Velumani, 2015)**.

Galaxaura elongate shows inhibition against *Escherichia coli* and *Klebsiella pneumoniae* **(Neveen *et al*., 2013)**. The essential oil of *Mentha piperita* shows fungal inhibition against *Aspergillus niger*, *Aspergillus flavus*, *Candida tropicalis* and *Candida albicans* **(Thanighaiarassu *et al*., 2014)**. *Pergularia daemia*, inhibits *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* **(Senthilkumar *et al*., 2017)**. Manuka Honey inhibits *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* **(Meenakshi and Jangir, 2016)**. *Zoogloea ramigera* shows antibacterial inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus* pyogenes **(Srivastava and Mausumi, 2014)**.

1.4.2 Reduced graphene oxide

Graphene is one of the carbon materials with multi-functionalized sp^2 hybridized carbon atoms in their structure. The graphene-based materials have potential applications in many fields due to their physical, chemical, biocompatibility, and biological properties. The targeting and drug delivery property of graphene is increased due to this chemical structure **(Sunny *et al*., 2016)**. Graphene used as an antimicrobial agent in surfaces and coating materials **(Soroush *et al*., 2016)**. It is used in optoelectronics touch screens, light-emitting devices, lasers and solar cell applications **(Hao and Zhou, 2014)**. Graphene and graphene oxide as a foundation of nanocomposites have garnered much interest among researchers in several fields **(Huifang *et al*., 2016)**.

Rice is one of the main food sources of life for man. Hence the need to give more importance to reduce infections in rice. *Xanthomonas oryzae* is a phytopathogenic bacterium responsible for infections in rice. The antibacterial potential of graphene against this bacterium exhibited with low dosage (250 µg/mL) and with high (94.48%) cell death but Bismerthiazol (common bactericide) showed only 13.3% mortality. The effect was caused by inactivating the bacteria and changes in the cell membranes **(Chen *et al*., 2013)**. This is because of the sharp edges in GO and the production of responsive oxygen species **(Chen *et al*., 2013; Gurunathan and Jin-Hoi, 2016)**. It showed antibacterial activity against *Pseudomonas aeruginosa* **(Sangiliyandi *et al*., 2012)**, *Bacillus cereus* and *Escherichia coli* **(Nagesh *et al*., 2015)**.

Reduced graphene oxide exhibits cellular uptake of MCF-7 and HER-2 cell lines **(Zheng *et al*., 2016)**, toxicity against HeLa cell lines **(Luo *et al*., 2017)** and A549 cell inhibition **(Khan *et al*., 2016; Tanveer *et al*., 2017; Kavinkumar *et al*., 2017)**. It is used to treat brain disorders **(Mendonca *et al*., 2015)** and it is also involved in antioxidant activities **(Kavyashree *et al*., 2016)**. It effectively delivers the drug for MRC-5 and Nasopharyngeal cancer cells **(Geetha *et al*., 2017)**. It is used to detect Cervical cancer **(Wu Dan *et al*., 2016)** and Prostate cancer **(Vinod *et al*., 2014)**. Reduced graphene oxide improves the electrode's sensitivity for 4-nitrophenol, and it applied in the Xiangjiang river to detect the real water samples **(Tang *et al*., 2013)**.

Chemical, electrochemical, and thermal methods are widely used to modify the graphene oxide (GO) into reduced graphene oxide (rGO). For example, hydrazine and its derivatives act as reducing agents in chemical synthesis, but these are highly toxic and explosive **(Lee and Kim, 2014)**. Using plant extracts helps to replace this difficulty due to the less toxic nature, extensive availability, and renewability. The plants provide advantages such as avoid clusters between sheets or flakes of carbon and hydrophilic content of rGO **(Mahata *et al*., 2018)**.

Polyalthia longifolia leaves aided reduced graphene oxide is used as a conductive transparent film **(Chamoli *et al*., 2016)**. *Ginkgo biloba* aided rGO shows biocompatibility for MDA-MB-231 **(Gurunathan *et al*., 2014)**. *Peltophorum pterocarpum* assisted rGO is involved in the electrochemical application **(Rahman *et al*., 2014)**. *Citrullus colocynthis* leaves aided rGO exhibits cytotoxicity against DU-145 cell lines **(Zhu *et al*., 2016)**. Prepared rGO using grapes, shows antimicrobial potential against *Escherichia coli* and *Staphylococcus aureus* and anti-proliferative activity against HCT-116 cell lines **(Yaragalla *et al*., 2016)**. *Vitis vinifera* aided rGO removes organic dyes from wastewater **(Upadhyay *et al*., 2015)**. Synthesized rGO using *Platanus orientalis* leaves shows cytotoxicity against cardiac cell lines **(Xing *et al*., 2016)**.

1.4.3 Nanocomposite

In this study, the synthesis of gold nanoparticles (AuNPs), reduced graphene oxide (rGO) and AuNPs-rGO composite has been attempted in a bio-mediated way. In this regard, the importance of nanocomposites is discussed in this section.

The properties and activity of individual particles may differ in the form of composite. Mixing of more than one material known as composite and enhancing the properties of individual one and providing better results. Nanocomposites depend not only on their individual but also on their morphology and interfacial character. It improves strength, stability, conductivity, chemical resistance, and thermal stability. It decreases the permeability of gas, hydrocarbon, and water **(Mousumi Sen, 2020)**. Nanocomposites provide important applications in many fields such as medical, electronics, automobiles, polymer technology, optical, sensor, *etc*.

The gold nanoparticle-graphene composite synthesized using nisin peptides shows cell inhibition (~80%) against MCF-7 cell lines at 10 µg/mL **(Otari *et al*., 2017)**. Glassy carbon electrode modified by Ethylene glycol-AuNPs-rGO is used in sulphite oxidation process. The electrochemical and electrocatalytic behaviour of this process is analyzed by electrochemical impedance spectroscopy and cyclic voltammetry studies **(Yu Hao *et al*., 2017)**. A biosensor was fabricated using rGO and AuNPs to detect the C-reactive protein. The composite of gold nanoparticles and graphene is deposited on the ITO modified electrode, and this prepared nano biosensor has high-sensitivity for protein (0.06 or 0.08 ng mL^{-1}) **(Yagati *et al*., 2015)**. AuNPs-rGO shows the catalytic impact for glucose detection (sensitivity- 34 mA M^{-1} cm^{-2} and -0.3V detection capability and Ag/AgCl was used for the comparison) and it also exhibits catalytic effect for H_2O_2 **(Bai and Shiu, 2014)**.

Xanthium strumarium plant extract was used to synthesis graphene and gold nanoparticle composite by sonication method and it suggests biomedical applications **(Wen *et al*., 2017)**. Seed mediated rGO and gold nanocomposite is used in drug release of doxorubicin (an anticancer drug). The optical properties of this nanocomposite were controlled without any surfactant and polymer stabilizers **(Wang *et al*., 2014)**. The photosensitivity of the Au-rGO composite is evaluated by fabricating the thin film as a visible light detector. This study improves the photoinduced carrier transfer, and the photocurrent increases with illumination and decreases in the dark **(Tjoa *et al*., 2012)**. Reduced graphene oxide coated gold nano-shells and nano-rods are enhancing the photothermal effect and this composite kills the cells rapidly than uncoated rGO **(Lim *et al*., 2013)**. Enzyme dependent reduced graphene oxide and gold nanoparticle composite show cytotoxicity against A-549 and HCT-116 cell lines in a dose-dependent manner **(Maddinedi *et al*., 2017)**.

Cadmium, Lead, Copper and Mercury are detected with the help of highly sensitive rGO supported AuNPs prepared using *Abelmoschus esculentus* extract. The sensitivity of nanocomposite modified electrode is 31.81, 12.69, 27.42 and 20.70 nM for $Cd^{2+,}$ Pb^{2+}, Cu^{2+} and Hg^{2+} respectively. The heavy metals are removed using bacteria (*Pseudomonas aeruginosa*, *Rhizobium gallicum*, *Staphylococcus aureus*, and *Bacillus subtilis*) by bioremediation **(Gnanaprakasam *et al*., 2016)**. Ascorbic acid aided AuNPs-rGO shows biocompatibility to HeLa cell lines and antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. This will cause cell death of microorganisms by the spillage of sugars and proteins from the cell layer **(Hussain *et al*., 2014)**.

The synthesis of gold nanoparticles, reduced graphene oxide, and their composite has been attempted by an eco-friendly method in the current investigation. The nanoparticles occupy an unremovable position in pharmacological research, and this prompted the preparation of these nanoparticles using selected samples.

1.5 Biodegradable film for skin substitute

Skin is affected by various factors such as infection, wound, pollution, burn injuries, cancer, *etc*. The replacement of damaged skin is a crucial and challenging one.

In worldwide, people are facing chronic (~300 million) and traumatic (~100 million) wound problems **(Das and Baker, 2016)**. Each year, in the US, 1.5 million burn injuries and 5000-12000 deaths caused **(Wolf *et al*., 1997)**. These effects are rectified by using artificial skin substitute, plastic surgery, skin transplantation and tissue engineering. Skin substitutes are a group of natural and synthetic elements responsible for a permanent or temporary effect. The application of skin substitutes started in the 15th century **(Halim *et al*., 2010)**. There are numerous synthetic skin available in the market (EpiDerm^{TM}, AlloDerm^{TM}, Epidex^{TM}, *etc*.,) and many types of research are still in progress to achieve the equivalent of normal skin **(Siti and Jamaluddin, 2014)**.

Mainly, skin substitutes are used in cosmetics, skin graft materials and act as a wound healer. Electronic skin is used for sensing environmental condition (strain, temperature, and pressure humidity) in robotics **(Dolbashid *et al*., 2017)**. Stem cell-based therapies are available to protect from infection and cure wound but are unable to have sensibility and thermoregulation capacities **(Dixit *et al*., 2017)**. Carbon nanotubes aided artificial skin is used to sense the environmental pressure, and is suggested in robotics **(Xin Ma *et al*., 2018)**. Plastic surgery is a common technique of autogenous skin graft, but the available material from a donor is limited **(Ferreira *et al*., 2011)**.

EDC treated chitosan-gelatin scaffold possesses porous structure, viscoelasticity, and mechanical properties. It showed good biocompatibility, and is a potential agent for cartilage tissue engineering **(Zhao *et al*., 2014)**. Gelatin increase the gelation and these hydrogels are suitable for potential application in tissue engineering of IVD **(Cheng *et al*., 2010)**. The presence of silica in scaffolds decreases the swelling ability and increases mechanical strength, leading to tissue engineering applications **(Kusumastuti *et al*., 2018)**. Alginate-gelatin crosslinked hydrogels indicated higher debasement and better help for fibroblasts connection, spreading, proliferation and viability. These hydrogels are suitable substance in the application of tissue-building and recovery **(Sarker *et al*., 2014)**.

Skin substitute prepared using mPEG–PCL–grafted gelatin/ hyaluronan/ chondroitin sulphate/ sericin shows *in vitro* biocompatibility with human keratinocytes, human fibroblasts, and human mesenchymal stem cells (hMSCs). It acts as an admirable dermal substitute for improving the healing activity on 2nd degree burn wounds **(Bhowmick *et al*., 2018)**. Fibrin–chitosan–sodium alginate composite sheet has high mechanical properties because of the most potent ionic interaction between amine and carboxyl groups of chitosan and alginate. The prepared skin substitutes applied on wounded dogs to analyze their potential in wound dressing application revealed good potency for wound dressing materials **(Devi *et al*., 2012)**.

Biopolymers (chitosan, alginate, gelatin, *etc*.,) are more efficient agents for skin regeneration **(Beatriz *et al*., 2018)**. Electro-spinning chitosan is used for wound dressing **(Claudia *et al*., 2018)**, chitosan-pectin-alginate scaffold shows antibacterial activity against *Escherichia coli* and *Staphylococcus epidermidis*. It does not show any cytotoxicity in *in vitro* studies and it reveals the chitosan-based materials is a potential candidate for tissue engineering **(Archana *et al*., 2013)** with low mechanical strength **(Maria, 2018)**.

Nanomaterials are effectively used in many clinically approved wound healing materials in the form of hydrogels, films, foams, wafers, sealants, and dressing materials. Nanomaterials are promising agents for enhancing wound care **(Das and Baker, 2016)**. The drug-loaded nanoparticle improves the efficacy of the treatment, improves the regeneration of skin tissue, and targeting drug delivery to the dermis **(Berthet *et al*., 2017)**. Due to the increase in the concentration of chitosan, the time of clotting is decreased. The decrease in the concentration of sodium and calcium alginate significantly reduces the clotting time. Compared with biopolymers, the nano biopolymer exhibiting less clotting time **(Sivakumar *et al*., 2017)**.

An artificial skin prepared using nano-TiO_2–chitosan with natural polymers showed moderate water absorptivity, fine thickness, low density and time-dependent biodegradability. It showed bactericidal property and better, faster recovery of the wound. The results suggest that prepared skin is a promising material in artificial skin substitutes **(Peng *et al*., 2008)**. Nano chitosan loaded calcium alginate hydrogel accelerates wound healing activity, showing antibacterial activity on *Escherichia coli* and *Staphylococcus aureus*. *In vivo* investigations on wounds in the skin of mice indicate advanced proliferation and relocation of VEC induced by the creation of ROS; accordingly, it prompts the fast-wound healing and re-epithelialization on the skin **(Wang *et al*., 2017)**.

In this study, biodegradable skin substitutes would be prepared, and plant-aided gold nanoparticles would be incorporated into skin substitutes. An attempt has been taken to fabricate gold nano-aided skin substitutes and to evaluate their various biological activity.

1.6 Objectives of the study

Cyperus rotundus (tuber) and *Hemigraphis alternata* (leaves) are traditionally used in medicines and possess various applications. Pumice stone is also used for cosmetic purposes from ancestral times. The depilation activity and the medicinal values of selected samples in skin substitutes were scientifically validated in this study entitled "**Traditional painless depilatory agents, their nanoparticle-incorporated biodegradable skin substitutes and their potential application in biological activities**". The present study is divided into three phases, and the objectives of the phases are given below.

Objectives of Phase I

- To evaluate the previous work on hair removal methods, selected samples, plant-based nanoparticles, and different skin substitutes.
- To prepare the different solvent extracts of *Cyperus rotundus* (tuber), and *Hemigraphis alternata* (leaves) by refluxing method and the pumice stone by the sonication and homogenization methods.
- To analyze the essential secondary metabolites in all extracts by phytochemical colour tests.
- To synthesize gold nanoparticles using aqueous extract of *Cyperus rotundus*, pumice stone and *Hemigraphis alternata* by room temperature, sonication, sunlight irradiation and microwave heating method.
- To optimize the formation of gold nanoparticle synthesis by concentration variation study.
- To prepare reduced graphene oxide using plant extract by refluxing method.
- To prepare nanocomposite using gold nanoparticles and reduced graphene oxide.
- To characterize the synthesized AuNPs, rGO and AuNPs-rGO composite by UV-Visible spectroscopy, FTIR, XRD, FESEM, EDS and Mapping analysis; Raman spectroscopy and TGA analysis for reduced graphene oxide.
- To analyze the antibacterial activity against gram-negative and gram-positive bacteria for synthesized nanoparticles.
- To analyze the anticancer potential on skin cancer cell line (A431).
- To analyze the toxicity on normal cells (HEK-293) by cytotoxicity studies.

Objectives of Phase II

- To fabricate and standardize the *in vitro* glass depilation tester to evaluate the depilation time of prepared depilatory creams.
- To prepare the depilatory creams using selected samples.
- To prepare gold nanoparticles aided depilatory creams.
- To analyze the hair damage by prepared samples through FESEM analysis.
- To analyze the antibacterial activity against gram-positive and gram-negative bacteria.
- To analyze the antifungal activity of prepared depilatory creams against *Aspergillus flavus* and *Aspergillus fumigates*.
- To analyze the antioxidant potential of prepared depilatory creams through DPPH radical scavenging activity.
- To analyze the toxicity on normal and skin cancer cell lines by cytotoxicity studies.

Objectives of Phase III

- To prepare the biodegradable skin substitutes using naturally available biodegradable polymers such as chitosan, sodium alginate, gelatin and fenugreek seed.
- To prepared gold nanoparticle-incorporated skin substitutes.
- To evaluate the physical and mechanical properties of prepared skin substitutes.
- To characterize the prepared skin substitutes through FTIR, FESEM, 3D optical profilometry and TGA analysis.
- To evaluate the antifungal activity of prepared skin substitutes against *Aspergillus niger*.
- To analyze the antioxidant activity of prepared skin substitutes through DPPH radical scavenging activity.
- To evaluate cytotoxicity against skin cancer (A375) and normal cell lines (HEK-293).
- To evaluate the cell growth and cell attachment of normal cells on prepared skin substitutes.