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PREPARATION OF HERBAL SANITARY NAPKINS AND ITS EFFECTIVENESS AGAINST MICROBES – A STUDY

A PROJECT REPORT

Submitted by

KADHIRVEL, R.

SANGLIRAJAN, G.P.

SILAMBARASAN, M.

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ANNA UNIVERSITY: CHENNAI 600 025

ANNA UNIVERSITY: CHENNAI 600 025

BONAFIDE CERTIFICATE

Certified that this project report "**PREPARATION OF HERBAL SANITARY NAPKINS AND ITS EFFECTIVENESS AGAINST MICROBES – A STUDY**" is the bonafide work of **KADHIRVEL, R., SANGLIRAJAN, G.P.,** and **SILAMBARASAN, M.** who carried out the project work under my supervision.

SIGNATURE

Dr.P.Rajasekaran

PROFESSOR AND HEAD

Department of Biotechnology,

Kumaraguru College of Technology,

Coimbatore – 641006.

SIGNATURE

Dr.S.Shanmugam

SUPERVISOR

ASSISTANT PROFESSOR

Department of Biotechnology,

Kumaraguru College of Technology,

Coimbatore – 641006.

CERTIFICATE OF EVALUATION

COLLEGE : Kumaraguru College of Technology

BRANCH : Biotechnology

SEMESTER : Eighth Semester

NAME OF THE STUDENTS	TITLE OF PROJECT	NAME OF THE SUPERVISOR WITH DESIGNATION
KADHIRVEL.R Reg No: 71205214009	**PREPARATION OF HERBAL SANITARY NAPKINS AND ITS EFFECTIVENESS AGAINST MICROBES – A STUDY**	**Dr.S.SHANMUGAM** Assistant Professor, Department of Biotechnology.
SANGLIRAJAN.G.P Reg No: 71205214024		
SILAMBARASAN.M Reg No: 71205214033		

The report of the project work submitted by the above students in partial fulfillment for the award of Bachelor of Technology degree in Biotechnology of Anna University was confirmed to be the report of the work done by the above students and then evaluated.

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[Kadhirvel.R]

[Sanglirajan.G.P]

ABSTRACT

There are problems associated with conventional napkins. Used napkins infect the environment with several pathogenic bacteria and fungi, since they do not have any antimicrobial agent in them. Women experience vulvar itching and burning. They also change the normal microflora of vagina and create bad odors. In this project, a new type of cost effective and eco friendly herbal (*Azadirachta indica* and *Curcuma longa*) and its effectiveness is tested against range of bacteria (*Staphylococcus aureus, Klebsiella sp, and Proteus vulgaris*) and fungi (*Trichophyton sp., Microsporum sp., Candida albicans*). In addition, effect of physical agents (UV irradiation and autoclave) on sanitary napkins will also be studied. The extract of neem and turmeric leaves is prepared using petroleum ether and optimized using orthogonal analysis. The optimal condition for neem extract was found to be 60°C, dilution ratio-1:10 kept for 3 hrs. The optimal condition for turmeric extract was found to be 65°C, dilution ratio-1:10 kept for 2 hrs. The antimicrobial activity is tested using broth dilution test and agar diffusion test. The napkin is modified by placing coir mat in between the wood pulp and its blood holding capacity was 72ml. The cloth is treated with optimized herbal extracts of neem and turmeric and tested for its effectiveness at different time intervals.

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LIST OF ABBREVIATIONS

MIC	Minimum Inhibitory Concentration
MLC	Minimum Lethal Concentration
ZOI	Zone Of Inhibition
UVGI	UltraViolet Germicidal Irradiation
PMT	Pre-menstrual Tension
PMS	Premenstrual Syndrome

INTRODUCTION

1. INTRODUCTION

A sanitary napkin is an absorbent item worn by a woman while she is menstruating, recovering from vaginal surgery, for lochia (post birth bleeding), abortion, or any other situation where it is necessary to absorb the flow of menses from one's vagina.

Through the ages women have used different forms of menstrual protection. Women often used strips of folded old cloth (rags) to catch their menstrual blood, which is why the term "on the rag" is used to refer to menstruation. Menstrual pads are made from a range of materials, differing depending on style, country of origin, and brand. Generally, sanitary napkins consist of a top sheet, a diffusion layer, an absorption layer, a back panel, and adhesive tape to prevent shifting. In addition, flaps or 3D side gathering has been added.

There are few major problems associated with current sanitary napkins because they do not have any antimicrobial agents incorporated in them. Used napkins infect the environment with several pathogenic bacteria and fungi, since they do not have any antimicrobial agent in them. Women experience vulvar itching and burning. They also change the normal microflora of vagina and create bad odors.

In this project, a new type of cost effective and eco friendly herbal sanitary napkins will be developed which will have a unique, fine herbal membrane with an optimized formula to accomplish many things such as release of itching, swelling and inflammation, protection from bacterial and fungi infections, removal of odors and normalization of vaginal microflora during menstruation. In addition, effect of physical agents on sanitary napkins

LITERATURE REVIEW

2. LITERATURE REVIEW

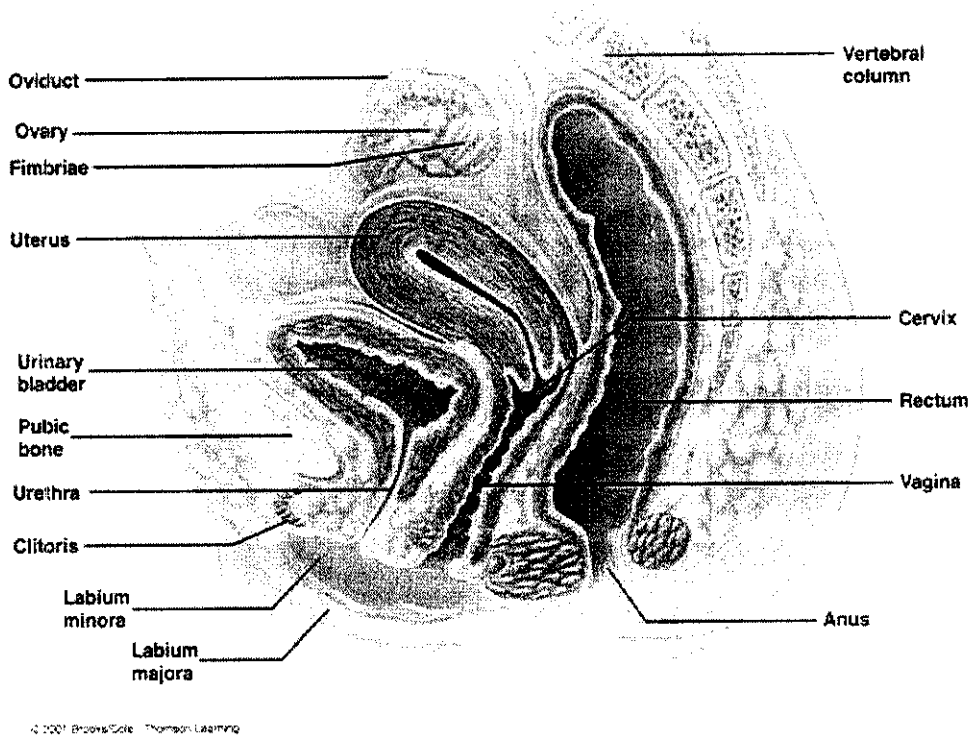
2.1 FEMALE REPRODUCTIVE SYSTEM

The human female reproductive system contains two main parts: the vagina and uterus, which act as the receptacle for the male's sperm, and the ovaries, which produce the female's ova. All of these parts are always internal; the vagina meets the outside at the vulva, which also includes the labia, clitoris and urethra. The vagina is attached to the uterus through the cervix, while the uterus is attached to the ovaries via the Fallopian tubes. At certain intervals, the ovaries release an ovum, which passes through the fallopian tube into the uterus.

If, in this transit, it meets with sperm, the sperm penetrate and merge with the egg, fertilizing it. The fertilization usually occurs in the oviducts, but can happen in the uterus itself. The zygote then implants itself in the wall of the uterus, where it begins the processes of embryogenesis and morphogenesis. When developed enough to survive outside the womb, the cervix dilates and contractions of the uterus propel the fetus through the birth canal, which is the vagina.

The ova are larger than sperm and are generally all created by birth. Approximately every month, a process of oogenesis matures one ovum to be sent down the Fallopian tube attached to its ovary in anticipation of fertilization. If not fertilized, this egg is flushed out of the system through menstruation.

Fig.2.1.1 Location of female genitourinary flora



2.2 MENSTRUAL CYCLE

2.2.1 MENSTRUATION

The menstrual cycle is the preparation of a woman's body for a possible pregnancy. This series of events occurs monthly during the woman's reproductive years (from puberty to menopause). The menstrual cycle usually lasts about 25 to 32 days. However, women's menstrual cycles vary in their length and amount of bleeding, according to the woman's age, weight, diet, amount of physical activity, level of stress and genetics. The length of the menstrual cycle is counted from the first day of menstrual bleeding until the day before the first day of the next menstrual bleeding. (Grace Mtawali *et al 1997*)

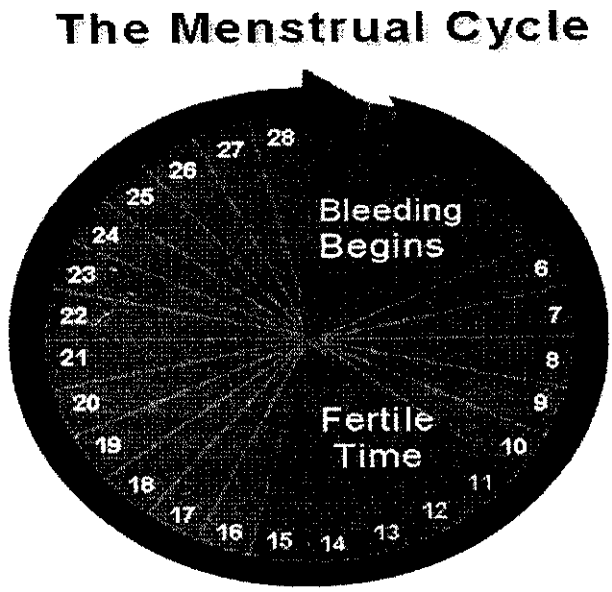
Eumenorrhea denotes normal, regular menstruation that lasts for a few days (usually 3 to 5 days, but anywhere from 2 to 7 days is considered normal). The menstrual fluid is largely a mixture of blood and tissue from the uterine lining (endometrium). The average blood loss during menstruation is 35 ml with 10–80 ml considered normal. Very little flow (less than 10ml) is called *hypomenorrhea*. Regular cycles with intervals of 21 days or fewer are *polymenorrhea*; frequent but irregular menstruation is known as *metrorrhagia*. Sudden heavy flows or amounts in excess of 80 ml are termed *menorrhagia*. Heavy menstruation that occurs frequently and irregularly is *menometrorrhagia*. The term for cycles with intervals exceeding 35 days is *oligomenorrhea*. Amenorrhea refers to more than three to six months without menses (in the absence of pregnancy) during a woman's reproductive years.

An enzyme called plasmin inhibits clotting in the menstrual fluid. Cramping in the abdomen, back, or upper thighs is common during the first few days of menstruation. The end of a woman's reproductive phase is called the menopause, which commonly occurs somewhere between the ages of 45 and 55. Medical problems such as anemia, infertility, and premenstrual syndrome (PMS) which is sometimes called Pre-menstrual Tension (PMT), can be due to disorders of the menstrual cycle and hormone imbalances.

The cyclic reproductive changes of the human female is marked by menstruation, during which some cells, unclotted blood from ruptured blood vessels, other fluids and the uterine endometrium is released through the cervix and vagina. Each menstrual cycle occurs about every 28 days and lasts 4-5 days. The menstruation occurs 12 to 14 days after an ovum is released from the ovary (ovulation), about once in four weeks. The periodicity of the cycle varies with different individuals. After fertilization, menstruation ceases, and is the first indication of pregnancy. The length of a woman's

cycles. A woman who experiences variations of less than eight days between her longest cycles and shortest cycles is considered to have regular menstrual cycles. It is unusual for a woman to experience cycle length variations of less than four days. Length variation between eight and 20 days is considered moderately irregular. Variation of 21 days or more between a woman's shortest and longest cycle lengths is considered very irregular.

Fig.2.2.1.1: The Menstrual cycle.



The menstrual cycle (of 28 days) is generally divided into four phases, with major events occurring in each phase. The 4 phases are:

(1) The menstrual (destructive) phase,

(2) The proliferative (follicular) phase,

(3) The ovulatory phase, and

(4) The secretory (luteal) phase

Table 2.2.1.1 Different phases in menstrual cycle

Name of phase	Average start day assuming a 28-day cycle	Average end day
menstrual phase	1	4
follicular phase (also known as proliferative phase)	5	13
ovulation phase	14	14
luteal phase (also known as secretory phase)	15	26
ischemic phase	27	28

1) **The menstrual phase**. The uterus lining (i.e., endometrium) and its blood vessels slough off , and is discharged with blood, mucus, cell debris and other fluid as the menses , through the vagina. This may last for 4-5 days. Menses occur when fertilization does not take place.

2) **The follicular phase** occurs between the end of menses, and ovulation. This phase cycle days 6 to 13 or 14 in a 28 day cycle. This phase is also called the *proliferative phase* because a hormone causes the lining of the uterus to grow, or proliferate, during this time.

Through the influence of a rise in follicle stimulating hormone (FSH) during the first days of the cycle, a few ovarian follicles are stimulated. These

part of a year in a process known as folliculogenesis, compete with each other for dominance. Under the influence of several hormones, all but one of these follicles will stop growing, while one dominant follicle in the ovary will continue to maturity. The follicle that reaches maturity is called a tertiary, or Graafian, follicle, and it forms the ovum.

As they mature, the follicles secrete increasing amounts of estradiol, an estrogen. The estrogens initiate the formation of a new layer of endometrium in the uterus, histologically identified as the proliferative endometrium. The estrogen also stimulates crypts in the cervix to produce fertile cervical mucus, which may be noticed by women practicing fertility awareness.

3) **The ovulatory phase** indicates the rupture of the Graafian follicle and release of the ovum (ovulation). It occurs some 14 days after the start of menstruation. During this phase the concentration of estrogen is high in blood and it stimulates the ovulation. The blood vessels enlarge and grow in the endometrial wall, and some secretory cells or glands are formed.

When the egg has nearly matured, the level of estradiol in the body has increased enough to trigger a sudden release of luteinizing hormone (LH) from the anterior pituitary gland. In the average cycle this LH surge starts around cycle day 12 and may last 48 hours. The release of LH matures the egg and weakens the wall of the follicle in the ovary, causing the fully developed follicle to release its secondary oocyte. The secondary oocyte promptly matures into an ootid and then becomes a mature ovum. The mature ovum has a diameter of about 0.2 mm.

After being released from the ovary, the egg is swept into the fallopian tube by the fimbria, which is a fringe of tissue at the end of each fallopian tube. After about a day, an unfertilized egg will disintegrate or dissolve in the

Fertilization by a spermatozoon, when it occurs, usually takes place in the ampulla, the widest section of the fallopian tubes. A fertilized egg immediately begins the process of embryogenesis, or development. The developing embryo takes about three days to reach the uterus and another three days to implant into the endometrium. It has usually reached the blastocyst stage at the time of implantation.

In some women, ovulation features a characteristic pain called *mittelschmerz* (German term meaning *middle pain*). The sudden change in hormones at the time of ovulation sometimes also causes light mid-cycle blood flow.

(4) **The secretory phase** occurs between ovulation and the onset of menses, i.e., the phase lasts about 14 days (cycle days 15 to 28). The endometrium which is under the influence of progesterone and estrogen, increases in size, becomes thick, the endometrial glands become enlarged, undergo maximum secretory activity and its blood vessels become coiled and enlarged.

Stimulated by gradually increasing amounts of estrogen in the follicular phase, menses slow then stop, and the lining of the uterus thickens. Follicles in the ovary begin developing under the influence of a complex interplay of hormones, and after several days one or occasionally two become dominant (non-dominant follicles atrophy and die). Approximately mid-cycle, 24-36 hours after the Luteinizing Hormone (LH) surges, the dominant follicle releases an ovum, or egg in an event called ovulation. After ovulation, the egg only lives for 24 hours or less without fertilization while the remains of the dominant follicle in the ovary become a corpus luteum; this body has a primary function of producing large amounts of progesterone. Under the influence of progesterone, the endometrium (uterine lining) changes to

implantation does not occur within approximately two weeks, the corpus luteum will involute, causing sharp drops in levels of both progesterone and estrogen. These hormone drops cause the uterus to shed its lining in a process termed menstruation.

2.3 SANITARY NAPKINS

2.3.1 HISTORY OF SANITARY NAPKIN

Table 2.3.1.1 History of Sanitary napkins

YEARS	CHANGE OF SANITARY PRODUCTS
1960	Cotton and cloth were used as absorbents
1961	Introduction of sanitary napkins in Japan
1965	Introduction of both side cut, nonwoven roll-up type
1968	Fluff pulp was used as an absorbent and wet-produced nonwoven cloth was used as the top
1974	Introduction of adhesive tape for slippage prevention
1976	Dry-produced nonwoven cloth, span bond, was used as the top sheet. Products became thinner and more compact.
1978	A superabsorbent polymer was used as an absorbent for the first time. It is two years prior to the introduction of disposable diapers for children.
1982	Introduction of 3D cut, and increased variety of adhesive tape positions

1985	Introduction of longer-size products for long-term and nighttime use
1986	Market was activated by the introduction of a new surface-treated polyethylene dry mesh
1987	Introduction of a flapper-type that does not slip or leak
1989	Introduction of an ultra thin product 2 mm thick
1993	Adoption of 3D side gathering
1994	A special sheet was added between the top sheet and the absorbent. They are made into a single part by quilting process.
1994	Introduction of a napkin with presslines to prevent shifting of the napkin by adding gap-like lines
1996	Introduction of a curved napkin emphasizing good fit

The first of the disposable pads were generally in the form of a cotton wool or similar fibrous rectangle covered with an absorbent liner. The liner ends were extended front and back so as to fit through loops in a special girdle or belt worn beneath undergarments. This design was notorious for slipping either forward or back of the intended position. The first commercially available American disposable napkins were Lister's Towels created by Johnson and Johnson. Disposable pads had their start with nurses using their wood pulp bandages to catch their menstrual flow, creating a pad that was made from easily obtainable materials and inexpensive enough to throw away after use. Several of the first disposable pad manufacturers were

also manufacturers of bandages, which could give an indication of what these products were like.

Prior to the 1950s, cotton or cloth was used to cope with menstrual blood. Sanitary napkins have undergone a revolutionary change since they were introduced in Japan in 1961. It has successfully changed the previously held negative attitudes of Japanese women regarding menstruation. There were a number of technological developments during the 35-year history of the sanitary napkin as listed in Table 2.3.1.1. Many of the technological developments during the 35 years occurred as women became more active, which created the demand for a sanitary napkin that afforded comfort and convenience. The result has been a thinner, comfortable product that could be used for a longer time. One such technological development, which deserves special attention, occurred around 1978 when superabsorbent polymers were used for sanitary napkins. As a result, the thickness of sanitary napkins was reduced to less than half that of the original products and performance was also drastically improved.

Later an adhesive strip was placed on the bottom of the pad for attachment to the saddle of the panties, and this became a favoured method with women. The belted sanitary napkin quickly became unavailable after the mid-eighties.

The design and materials used to make pads also changed through the 1980s to today. With earlier materials not being as absorbent and effective, and early pads being up to two centimetres thick, leaks were a major problem. Some variations introduced were quilting of the lining, adding "wings" and reducing the thickness of the pad. The materials used to manufacture most pads are derived from the petroleum industry and forestry. The absorbent core, made from chlorine bleached wood pulp, could be reduced to make

slimmer products with the addition of polyacrylate gels which sucks up the liquid quickly and holds it in a suspension under pressure. The remaining materials are mostly derived from the petroleum industry; the cover stock used is polypropylene non woven, with the leak proof barrier made from polyethylene film. The problems with these materials are that they are neither biodegradable nor recyclable, so disposal issue are created worldwide, often with disposed products ending in the oceans of the world.

There are many styles of cloth menstrual pads available today. Popular styles of cloth menstrual pads include all-in-one, or AIO pads, in which the absorbent layer is sewn inside the pad, 'inserts on top' style pads, which have absorbent layers that can be secured on top of the pad as needed, envelope or pocket style pads, which have absorbent layers that can be inserted inside the pad as needed, and a foldable style, in which the pad folds around the absorbent layers.

Table 2.3.1.2 Different types of disposable menstrual pads

Panty Liner	Designed to absorb daily vaginal discharge, light menstrual flow, "spotting", slight urinary incontinence, or as a backup for tampon use.
Ultra-thin	A very compact (thin) pad, which may be as absorbent as a Regular or Maxi/Super pad but with less bulk.
Regular	A middle range absorbency pad.
Maxi / Super	A larger absorbency pad, useful for the start of the menstrual cycle when menstruation is often heaviest.
Night	A longer pad to allow for more protection while the wearer

Maternity	These are usually slightly longer than a maxi/Super pad and are designed to be worn to absorb lochia (bleeding that occurs after childbirth).

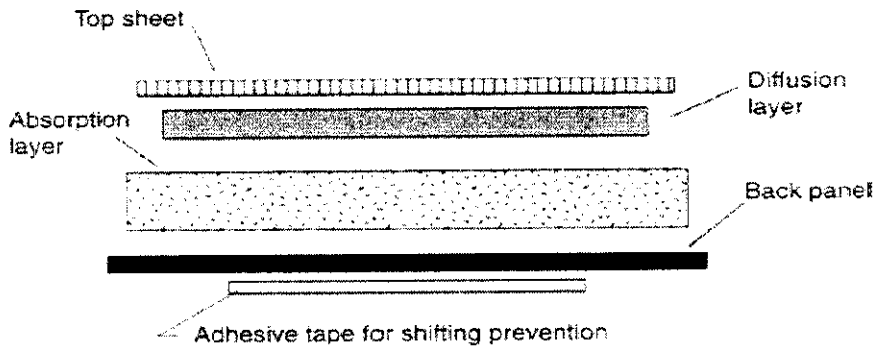
2.3.2 Worldwide Market

The worldwide market for sanitary napkins and disposable diapers for children and adults is approximately 30 billion dollars. Of this amount, the market for sanitary napkins is about 10 billion dollars, around 33 % of all sanitary products. Based on the number of products worldwide, the total number of sanitary products comprises approximately 142 billion, which includes about 80 billion sanitary napkins (about 56 % of all sanitary products). Assuming that approximately half the world population of 5.6 billion is women and about 50 % of the women are in the age range of 15 to 50 and are users of sanitary napkins, the world market will be 252 billion napkins if 100 % of those who need them, use them. At the present time, only less than 32 % of women in this age bracket use sanitary napkins. Hence, emphasis on market development is believed to shift to developing countries. The market share of sanitary napkins in the United States, Western Europe and Japan is nearly 100 % and no significant growth can be expected. Hence, even by introducing high value-added products, the future growth rate is expected to be around 1 to 3 %. On the other hand, in the developing countries of the Asia/Pacific region other than Japan and Latin American countries, the market share is low. Thus, it is expected that an annual growth of 10 to 15 % can be maintained until the present by cultivating new users. The major producers of sanitary napkins in the world are Procter & Gamble, Kimberly-Clark, Molnlycke/Peaudouce, Johnson and Johnson, Kao and Unicharm. In addition to these, various regional producers are joining the

2.3.3 STRUCTURE OF SANITARY NAPKIN

Functions that are required of sanitary napkins are: high absorbency/no leakage, no chafing, comfort, good fit to the body contour and no humidity that leads to skin rash. In order to improve on these functions, there have been many technological developments over the 35-year history of sanitary napkins. Currently, sanitary napkins consist of a top sheet, a diffusion layer, an absorption layer, a back panel, and adhesive tape to prevent shifting. In addition, flaps or 3D side gathering has been added. Nonwoven cloth was once the material of choice for the top sheet. In 1986, a new material, mesh sheet, was introduced and gained rapid popularity with young users. At present, the mesh sheet has completely replaced the nonwoven cloth. Based on numbers, the mesh sheet now occupies more than 60 % of the market. The mesh sheet is a polyethylene sheet with funnel-like holes. The polymer surface is treated by a surfactant to make it hydrophilic, thus blood does not adhere and its dryness and cleanliness are popular features among users. In particular, a dry mesh sheet, a 3D polyethylene film with holes, exhibits no obvious sign after use, making it an attractive feature for the consumers.

Fig 2.3.3.1 Structure of sanitary napkin



The role of the diffusion layer is to quickly accept the bodily fluid from the top sheet and transfer it to the absorption layer. As the diffusion layer, tissue, high-loft nonwoven cloth, or pulp laminate is used. Fluff pulp or crepe pulp was used as the absorbent prior to 1978. Today, materials with a superabsorbent polymer dominate the choice of the material. There are basically two types of absorption layers---either a laminate in which a superabsorbent polymer is sandwiched by a tissue or nonwoven cloth, or a mixture of a superabsorbent polymer and pulp wrapped by a tissue around a nonwoven cloth. In addition, there are variations of these two of absorption layers. For example, a transport layer is introduced between the absorption core and the back panel. Furthermore, a small-sized laminate with a high polymer concentration can be placed on top of the absorption core. The amount of a superabsorbent polymer used for a sanitary napkin is approximately 0.6 to 1.5 g. The back sheet is a water- nonpermeable sheet which makes contact with the clothing. It is mainly made of a polyethylene film. A laminate of polyethylene film and nonwoven cloth is also used as a cloth like back panel.

There are many kinds of sanitary napkins depending on the style of side characteristics, size of product, and thickness. The side characteristics can be classified as 3D side gathering or flap-types. The lengths are classified as regular (20mm), long (26-30mm), and extra-long (33mm); with regard to thickness, these are thick, thin, and ultra-thin products. The thick napkin uses a mixed core consisting of a superabsorbent polymer and pulp, its total thickness is approximately 10mm. The ultrathin napkin has a laminated absorption core and its thickness is at most 2 mm. The thin napkin has a thickness of 4 to 6 mm, and is the intermediate-sized napkin. Many consumers use different products depending on regular use, night time use or extended use.

Approximately 10 % of the users have experienced some type of leakage problems resulting in soiled clothes (Eason, 1996). This indicates that leakage protection, the most fundamental function of sanitary napkins, is not yet sufficient. Thus it is necessary to improve the absorbency of the absorption core to improve the protection function. A superabsorbent polymer that has a high absorbency towards blood, the so-called menses-specific superabsorbent polymer, is essential for a high-performance core and its development specific is strongly desired.

2.4 PHYSICAL AGENTS

2.4.1 UV RADIATION

Ultraviolet germicidal irradiation (UVGI) is a sterilization method that uses ultraviolet (UV) light at sufficiently short wavelength to break down micro-organisms. It is used in a variety of applications, such as food, air and water purification. UV has been a known mutagen at the cellular level for more than 100 years. The 1903 Nobel Prize for Medicine was awarded to Niels Finsen for his use of UV against tuberculosis.

UVGI utilises the short wavelength of UV that is harmful to forms of life at the micro-organic level. It is effective in destroying the nucleic acids in these organisms so that their DNA is disrupted by the UV radiation, which is a form of ionising radiation. This removes their reproductive capabilities and/or kills them.

The wavelength of UV that causes this effect is rare on Earth as its atmosphere blocks it. Using a UVGI device in certain environments like circulating air or water systems creates a deadly effect on micro-organisms

with a filtration system, UVGI can remove harmful micro-organisms from these environments.

The application of UVGI to sterilization has been an accepted practice since the mid-20th century. It has been used primarily in medical sanitation and sterile work facilities. Increasingly it was employed to sterilize drinking and wastewater, as the holding facilities were enclosed and could be circulated to ensure a higher exposure to the UV. In recent years UVGI has found renewed application in air sanitization.

Ultraviolet light is electromagnetic radiation with wavelengths shorter than visible light. UV can be separated into various ranges, with short range UV considered "germicidal UV." At certain wavelengths UV is mutagenic to bacteria, viruses and other micro-organisms. UV radiation is found effective in killing *Legionella* bacteria (*Peterson,* 2002, US Patent no 6770192).At a wavelength of 254 nm, UV will destroy the microorganisms, rendering them harmless or prohibiting growth and reproduction. It is a process similar to the UV effect of higher wavelengths on humans, such as sunburn or sun glare. Micro-organisms have less protection from UV and cannot survive prolonged exposure to it.

A UVGI system is designed to expose environments such as water tanks, sealed rooms and forced air systems to germicidal UV. Exposure comes from germicidal lamps that emit germicidal UV electromagnetic radiation at the correct wavelength, thus irradiating the environment. The forced flow of air or water through this environment ensures the exposure.

2.4.2 AUTOCLAVE

A widely-used method for heat sterilization is the autoclave. Autoclaves commonly use steam heated to 121 °C or 134 °C. To achieve

134 °C is required. Autoclave is more effective when the materials are wrapped in autoclavable covers which allow even temperature distribution throughout the material and therefore complete sterilization.

Proper autoclave treatment will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant. To ensure the autoclaving process was able to cause sterilization, most autoclaves have meters and charts that record or display pertinent information such as temperature and pressure as a function of time.

For effective sterilization, steam needs to penetrate the autoclave load uniformly, so an autoclave must not be overcrowder. During the initial heating of the chamber, residual air must be removed. Indicators should be placed in the most difficult places for the steam to reach to ensure that steam actually penetrates there.

For autoclaving, as for all disinfection of sterilization methods, cleaning is critical. Extraneous biological matter or grime may shield organisms from the property intended to kill them, whether it physical or chemical. Cleaning can also remove a large number of organisms. Proper cleaning can be achieved by physical scrubbing. This should be done with detergent and warm water to get the best results. Cleaning instruments or utensils with organic matter, cool water must be used because warm or hot water may cause organic debris to coagulate. Treatment with ultrasound or pulsed air can also be used to remove debris.

2.5 HERBALISM

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medicinal botany, herbal medicine, herbology, and phytotherapy.

plant parts in response to illness. The risk benefit ratio favored animals and protohumans that were inclined to experiment in times of sickness. Over time, and with insight, instinct, and trial-and-error, a base of knowledge would have been acquired within early tribal communities. As this knowledge base expanded over the generations, the specialized role of the herbalist emerged. The process would likely have occurred in varying manners within a wide diversity of cultures.

All plants produce chemical compounds as part of their normal metabolic activities. These include primary metabolites, such as sugars and fats, found in all plants, and secondary metabolites found in a smaller range of plants, some useful ones found only in a particular genus or species. The functions of secondary metabolites are varied. Plants up regulate and downregulate their biochemical paths in response to the local mix of herbivores, pollinators and microorganisms. The chemical profile of a single plant may vary over time as it reacts to changing conditions. It is the secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs.

Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs. Alkaloids contain a ring with nitrogen. Many alkaloids have dramatic effects on the central nervous system. Caffeine is an alkaloid that provides a mild lift but the alkaloids in datura cause severe intoxication and even death. Phenolics contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics. Terpenoids are built up from terpene building blocks. Each terpene consists of two paired isoprenes. The names monoterpenes, sesquiterpenes, diterpenes and triterpenes are based on the number of isoprene units. The

produce the reds, yellows and oranges of pumpkin, corn and tomatoes. Glycosides consist of a glucose moiety attached to an aglycone. The aglycone is a molecule that is bioactive in its free form but inert until the glycoside bond is broken by water or enzymes. This mechanism allows the plant to defer the availability of the molecule to an appropriate time, similar to a safety lock on a gun. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by an herbivore.

There is abundant evidence from epidemiological studies that the phytochemicals in fruits and vegetables can significantly reduce the risk of cancer, probably due to polyphenol antioxidant and anti-inflammatory effects. Phytochemicals have been used as drugs for millennia. For example, Hippocrates in 400 BC used to prescribe willow tree leaves to abate fever. Salicin, with potent anti-inflammatory and pain-relieving properties, was originally extracted from the White Willow Tree and later synthetically produced to become the staple over the counter drug called Aspirin. The number one drug for cancer worldwide Taxol (paclitaxel), is a phytochemical initially extracted and purified from the Pacific Yew Tree.

Some phytochemicals with potent medicinal properties may be elements, rather than complex organic molecules (Dewick, 2002). Selenium for example is abundant in Brassica vegetables which may have potent anti-viral and anti-cancer properties. In human clinical trials, selenium supplementation has been shown to reduce the HIV viral load and is currently being recommended worldwide by physicians as an adjuvant for AIDS treatments. It has also been shown to reduce mortality among prostate cancer patients. Selenium is a pre-cursor of Glutathione, a potent and important antioxidant manufactured primarily in the liver. There are currently many other phytochemicals with potent medicinal properties that are in clinical

clinical trials for cardiovascular diseases and prostate cancer. Human clinical trials have demonstrated that lycopene helps to improve blood flow through the heart and clinical studies suggest anti-cancer activity against prostate cancer. Lutein and zeaxanthin from spinach have been shown through clinical trials to directly improve human visual performance and help prevent the onset of macular degeneration and cataracts.

Many phytochemicals have anti-inflammatory properties. Inflammation is a factor in many diseases of aging including Alzheimer's and Arthritis, and many artificial anti-inflammatories have unfortunate side-effects. Turmeric is also reported to be active against skin cancer (Melanoma). In a landmark nutritional sciences study, scientists demonstrated that a diet rich in tomotoes and broccoli was more effective in inhibiting prostate cancer growth than a leading drug for prostate cancer.

Clinical investigations are ongoing worldwide on thousands of phytochemicals with medicinal properties (Kaufman *et al*, 1999). Fossils of plants date back as early as 3.2 billion years ago. These plants provided the foundation upon which animal life and later, human life were based on. They provide bodybuilding food and calories as well as vitamins essential for metabolic regulation. Plants also yield active principles employed as medicines. Finding healing powers in plants is an ancient idea. Hundreds, if not thousands, of indigenous plants have been used by people on all continents as poultices and infusions dating back to prehistory. There is evidence of Neanderthals, living 60 000 years ago in present-day Iraq, using hollyhock, which is still in medicinal use around the world today (Cowan, 1999).

The fall of ancient civilisations resulted in the destruction or loss of much of the documentation of plant pharmaceuticals but many cultures

continued in the excavation of the older works as well as building upon them. Native Americans were reported to have used 1625 species of plants as food while 2564 found use as drugs, while the Europeans started turning towards botanicals when treatment in the 1800s became dangerous and ineffective (Cowan, 1999). Today some 1500 species of medicinal and aromatic plants are widely used in Albania, Bulgaria, Croatia, France, Germany, Hungary, Spain, Turkey and the United Kingdom (Hoareau, 1999).

2.6 NEEM

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Meliceae

Genus: *Azadirachta*

Species*: indica*

Binomial name*: Azadirachta indica*

For centuries, the people of India have utilized the neem tree (Azadirachta indica) for its variety of medicinal uses. The importance of the neem tree has been recognized by US National Academy of Sciences, which published a report in 1992 entitled 'Neem – a tree for solving global problems'. The twigs, leaves and bark of the the neem tree provides so many benefits. Each part of the neem tree has some medicinal property. In India, the tree is variously known as "Divine Tree", "Heal All", "Nature's

Neem has two closely related species: *A. indica A. juss M.azedarac,* the former is popularly known as Indian neem (margosa tree) or Indian lilac. It is one of two species in the genus *Azadirachta,* and is native to Bangladesh, India, Myanmar, and Pakistan growing in tropical and semi-tropical regions. Other vernacular names include Azad Dirakht (Persian), DogonYaro (Nigerian), Margosa, Neeb (Arabic), Nimtree, Nimba (Sanskrit), Vepu, Vempu, Vepa (Telugu), Bevu (Kannada), Vembu (Tamil) and Arya veppu (Malayalam). In East Africa it is also known as Mwarobaini (Kiswahili), which means the tree of the 40; it's said to treat 40 different diseases. Neem has been extensively used in ayurveda, unani and homoeopathic medicine.The Sanskrit name of neem tree is *Arishtha* meaning 'reliever of sickness' and hence is considered as *Sarbaroganibarini*. The tree is still regarded as 'village dispensary' in India. The other as the Persian lilac.

Neem is a fast-growing tree that can reach a height of 15-20 m, rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. The branches are wide spread. The fairly dense grown is roundish or oval and may reach the diameter of 15-20 m in old, free-standing specimens. The trunk is relatively short, straight and may reach a diameter of 1.2 m. The bark is hard, fissured or scaly, and whitish-grey to reddish-brown. The sapwood is greyish-white and the heartwood reddish when first exposed to the air becoming reddish-brown after exposure. The root system consists of a strong taproot and well developed lateral roots. The alternate, pinnate leaves are 20-40 cm long, with 20-31 medium to dark green leaflets about 3-8 cm long. The terminal leaflet is often missing. The petioles are short. Very young leaves are reddish to purplish in colour. The shape of mature leaflets is more or less asymmetric and their margins are dentate with the exception of the base of their basiscopal half, which is normally very

axillary, normally more-or-less drooping panicles which are up to 25 cm long. The inflorescences, which branch up to the third degree, bear 150-250 flowers. An individual flower is 5-6 mm long and 8-11 mm wide. Protandrous, bisexual flowers and male flowers exist on the same individual (polygamous). The fruit is a glabrous olive-like drupe which varies in shape from elongate oval to nearly roundish, and when ripe are 1.4-2.8 x 1.0-1.5 cm. The fruit skin (exocarp) is thin and the bitter-sweet pulp (mesocarp) is yellowish-white and very fibrous. The mesocarp is 0.3-0.5 cm thick. The white, hard inner shell (endocarp) of the fruit encloses one, rarely two or three, elongated seeds (kernels) having a brown seed coat.

The neem is a tree noted for its drought resistance. Normally it thrives in areas with sub-arid to sub-humid conditions, with an annual rainfall between 400 and 1200 mm. It can grow in regions with an annual rainfall below 400 mm, but in such cases it depends largely on the ground water levels. Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils (pH 6.2-7.0). It is a typical tropical/subtropical tree and exists at annual mean temperatures between 21-32 °C. It can tolerate high to very high temperatures. It does not tolerate temperature below 4 °C (leaf shedding and death may ensue). Neem is a life giving tree in South India, especially for the dry coastal southern districts. It is one of the very few shade giving trees that thrive in the drought prone areas. The trees are not at all delicate about the water quality and thrive on the merest trickle of water, whatever the quality be. In Tamil Nadu it is very common to see neem trees used as shade giving trees lining the streets or in most people's back yards.

Kausik Biswas *et al* (2002) have recently reviewed the biological activities some of the neem compounds, pharmacological actions of the neem

with their safety evaluation. More than 135 compounds have been isolated from different parts of neem (Subapriya *et al 2005*) and several reviews have also been published on the chemistry and structural diversity of these compounds. The compounds have been divided into two major classes: isoprenoids (like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C- secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc.(Kausik Biswas *et al* 2002)

Traditionally, Indians bathed in neem leaves steeped in hot water. Since there has never been a report of the topical application of neem causing an adverse side effect, this is a common procedure to cure skin ailments or allergic reactions. Neem's function as a fungicide depends on the compounds gedunin and nimbidol in its leaf. It is noted by many observers to relieve athlete's foot, ringworm and yeast-like fungi that can develop internally. Neem also may provide anti-viral treatment for smallpox, chicken pox and warts--especially when applied directly to the skin. Its effectiveness is due in part to its ability to inhibit a virus from multiplying and spreading. In India and Africa, people use the twigs of the neem tree as toothbrushes. This practice has apparently influenced current dental products that incorporate neem bark extracts in tooth pastes and mouthwashes. Neem produces pain-relieving, anti-inflammatory and fever reducing compounds that can aid in the healing of cuts, burns, sprains, earaches and headaches, as well as fevers. Several studies of neem extracts in supressing malaria have been conducted, all supporting its use in treatment.

More research is being conducted in this area because of neem's widespread availability in overpopulated countries unable to afford pricier birth control methods.

Neem is an environmentalist's guilt-free natural product. The neem tree grows in abundance and is quite resilient to surrounding nature. Since it has been transplanted to Africa, the Middle East and South America, it has thrived in even the poorest of soils because of its ability to extract nutrients from the ground. Almost every part of the plant is used, adding to its overall efficiency. Neem's role as a wonder drug is traced as far back as 4,500 years ago. The earliest documentation of neem mentioned the fruit, seeds, oil, leaves, roots and bark for their adventageous medicinal properties. These benefits are listed in the ancient documents Caraka-Samhita and Susruta Samhita, tthe books at the foundation of the Indian system of natural treatment, ayurveda. Neem has a garlic like odor, and a bitter taste.

Perhaps neem's most touted advantage is the effect it has upon the skin. Preparations from the leaves or oils of the tree are used as general antiseptics, according to a report of The National Research Council's Adhoc Panel of the Board on Science and Technology for International Development. Due to neem's antibacterial properties, it is effective in fighting most epidermal dysfunctions such as acne, psoriasis and eczema. Ancient ayurvedic practitioners believed high sugar levels in the body caused skin disease; neem's bitter quality was said to counteract the sweetness.

Antibacterial Compounds in Neem - Ongoing research over the past 45 years recognizes these traditional uses of neem, but researchers typically list them as "known to be" rather than reporting on their action. More recent reports focus on antibacterial activities in the mouth, specifically gum disease and cavities, as well as preventing sexually transmitted diseases as a vaginal

contraceptive. *Azadirachta indica* extract had significant antibacterial activity against the multi-drug-resistant *Vibrio cholerae* .It is also effective against *Staphylococcus aureus* (Wafaa Helmy *et al* 2007)and the rate and extent of bacterial killing increases with concentration and time (Okemo *et al* 2001)

Antifungal Properties of Neem - Like neem's antibacterial properties, its antifungal properties are often a given among scientists in India and other Asian nations where most of do indicate that compounds in neem help control fungi that can cause athlete's foot, ringworm and *Candida*, the organism that causes yeast infections and thrush, as well as fungus that may affect plants (Charmaine Lloyd *et al* 2005).Neem is also found effective against *Trichophyton sp.* and *Microsporum sp.* (Natarajan *et al* 2003)

Anti-Inflammatory Properties of Neem - Nimbidin, a component of neem, has been show to possess potent anti-inflammatory activity in both in vivo and in vitro settings. Researchers suggest that nimbidin suppresses the functions of macrophages and neutrophils involved in inflammation.

Antioxidant Compounds in Neem - Oxidative stress, the process through which free radicals are created, is a normal function of the body but the resulting molecules are unstable and can damage other cells. Researchers have associated a series of disorders, including cardiovascular disease, eye health, cataracts and macular degeneration, age-related neurodegeneration (decline of the brain and nervous system) and even cancer with high levels of free radicals. Antioxidants, including those found in vitamins A, C and E, provide the free radicals with electrons to minimize damage. More than a dozen studies conducted in India,

Antiviral Compounds in Neem - Other researchers report that neem inhibits the growth of Dengue virus, a hemorrhagic fever and interferes with the reproduction of the coxsackie B virus, one of a group of "Enteroviruses" that are second only to the common cold as the most infectious viral agents in human beings. The compounds like Azadirachtin, salanin, numbin, meliantrial found to possess antiviral activity (Tipu *et al* 2006).

Cancer & Neem - More than two dozen studies, both in test tubes and on animals, document neem's efficacy in killing cancer cells or boosting the body's immune system to protect it from damage. Neem or its isolated compounds have shown impressive action against a wide variety of human cancer cell lines and in animal models for cancers that include colon, stomach, Ehrlich's carcinoma, lung, liver, skin, oral, prostate and breast cancers. Two separate reports indicate that it may be helpful in enhancing the activity and reducing side effects of some conventional cancer treatments.

Diabetes & Neem - With its extremely bitter properties, neem has been a cornerstone of Ayurvedic therapy or disorders. Some of the earliest reports on neem, indicated that insulin requirements could be cut. More recent studies have focused on animals, including one report which indicates that neem's hypoglycemic effect is comparable to the prescription drug glibenclamide and noted that it may be beneficial in preventing or delaying the onset of disease (Lans 2006).

Immunostimulatory Compounds In Neem - It boosts both the lymphocytic and cell-mediated systems, including "Killer T" cells which are able to destroy microbes, viruses and cancer cells by injecting toxic chemicals into the invaders.

Malaria & Neem - While questions still remain about the dosage required in human beings, neem clearly has great potential in preventing malaria, a parasite that kills more than a million people per year. Several in vitro studies indicate significant protection, including one that concluded it was more effective than chloroquine, a drug to which the parasite is becoming resistant. One interesting report indicates that it may increase the efficacy of chloroquine when the two are taken together.

Neuroprotective Effect of Neem - A single study shows that indicates that antioxidant compounds in neem helped to prevent brain damage in rats who had suffered a stroke by enhancing lipid peroxidation and increasing ascorbic acid (Vitamin C) in the brain.

Ulcers & Neem - One of the few recent clinical trials among humans using neem indicates that neem bark causes significant decreases in gastric acid secretion (77%), as well as gastric secretion volume (63%) and pepsin activity (50%) That research may be particularly important for people with arthritis or other chronic pain. Along with its own anti-inflammatory compounds, neem may help counteract the gastric damage caused by pain relievers like aspirin and ibuprofen.

Safety Issues & Neem - When used as directed, neem leaf and bark show very few signs of toxicity even at high levels. Neem oil, however, should not be used internally. Neem also contains compounds similar to aspirin and should never be used in children with colds, fevers or flu.

2.7 TURMERIC

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Zingiberales

Family: Zingiberaceae

Genus: *Curcuma*

Species: *longa*

Binomial name: *Curcuma longa*

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to tropical South Asia and needs temperatures between 20° C and 30° C, and a considerable amount of annual rainfall to thrive. Plants are gathered annually for their rhizomes, and re-seeded from some of those rhizomes in the following season.

The rhizomes are boiled for several hours and then dried in hot ovens, after which they are ground into a deep orange-yellow powder commonly used as a spice in curries and other South Asian and Middle Eastern cuisine, for dyeing, and to impart color to mustard condiments. Its active ingredient is curcumin and it has an earthy, bitter, peppery flavor and a mustardy smell.

It is only in recent years that Western scientists have increasingly recognised the medicinal properties of turmeric. In the latter half of the 20th century, curcumin was identified as responsible for most of the biological effects of turmeric. Turmeric is of great interest in the field of Neuroscience that one of its major functional chemical components enhances the production of BDNF (Brain Derived Neurotrophic Factor). Other BDNF enchancing factors include Glutamate, Exercise, Caloric Restriction, Intellectual Stimulation, and brain injury (as a compensatory mechanism).

In Ayurvedic practices, turmeric is thought to have many medicinal properties and many in South Asia (particularly India) use it as a readily available antiseptic for cuts, burns and bruises. Practitioners of Ayurvedic medicine say it has fluoride which is thought to be essential for teeth. It is also used as an antibacterial agent. It is taken in some Asian countries as a dietary supplement, which allegedly helps with stomach problems and other ailments. Indians use turmeric in a wide variety of skin creams that are also exported to neighboring countries. It is currently being investigated for possible benefits in Alzheimer's disease, cancer and liver disorders.

Turmeric has been valued as a health and beauty aid for thousands of years in India and the rest of the South East Asia region and hundreds of years in the Far East. The West is slowly coming appreciate it and no longer just look on it as "poor man's saffron". Excellent for the skin, turmeric powder is used in pastes and masks to lighten the skin, to prevent and cure pigmentation marks, relieve eczema, prevent dryness, prevent wrinkles, remove hair and heal spots. Applied to the hair will lighten blond hair and add highlights to brown hair. In first aid the powder can be applied to cuts as an antiseptic and to stop bleeding. Mixed in water and drunk it will relieve digestive problems like bloating and gas, sore throats and coughs. A paste made with chickpea flour, vegan yogurt with turmeric oil or powder, can be applied to sprains or arthritic joints to relieve pain. Mixed with aloe vera gel it can be applied to burns.

Besides flavoring food, to purify the blood and skin conditions remedy is probably the most common use of Turmeric in Ayurveda.

- The main organs that turmeric treats are the skin, heart, liver and lungs.

- Turmeric is used for epilepsy and bleeding disorders, skin diseases, to purify the body-mind, and to help the lungs expel Kapha.
- **Activities of Turmeric include:** Alterative, analgesic, antibacterial, anti-inflammatory, anti-tumor, anti-allergic, antiseptic (Rambir Singh *et al* 2002), antispasmodic, appetizer, astringent, cardiovascular, carminative, cholagogue, digestive, diuretic, stimulant, and vulnerary.
- **Therapeutic uses of Turmeric:** Anemia, cancer, diabetes, food poisoning, gallstones, indigestion, parasites, poor circulation, staph infections, and wounds.
- Dietary spice components of *Curcuma longa* and *Abroma augusta* have been screened for their protective effect against reactive oxygen species induced lipid peroxidation. They have been found to be efficient antioxidant when administered in combination (Halim Eshrat 2002).
- **Antibacterial activity:** Turmeric has antibacterial activity against *S.aureus, Proteus vulgaris, Klebsiella sp.* (Seher Gur *et al 2006*)
- **Antifungal activity**: Turmeric is effective against ***Trichophyton*** induced diseases.
- Turmeric helps to regulate the female reproductive system and purifies the uterus and breast milk, and in men it purifies and builds semen, which is counterintuitive for a pungent bitter.
- Turmeric reduces fevers, diarrhea, urinary disorders, and insanity, poisoning, cough, and lactation problems in general.
- Turmeric is used to treat external ulcers. Turmeric is used to remove watery discharges like leucorrhea, and any pus in the eyes, ears, or in wounds, etc.
- It is also an antioxidant Ayurveda recognizes turmeric as a heating spice, contributing bitter, pungent and astringent tastes.

- Turmeric is a great carminative, able to calm an upset digestive system by getting rid of gas and distention. Carminatives also tend to increase absorption and nurture the intestinal flora.
- In Ayurvedic cooking, turmeric is everywhere, this multifaceted wonder spice helps

  - ✓ Detoxify the liver
  - ✓ Balance cholesterol levels
  - ✓ Fight allergies
  - ✓ Stimulate digestion
  - ✓ Boost immunity
  - ✓ Enhance the complexion

2.7.1 TURMERIC OIL

1.	Origin/ Distribution	Because of ancient trade, the origin of Turmeric cannot accurately reconstructed, probably South East Asia or South Asia. Today, It is widely cultivated in India and South East Asia.
2.	Ethnomedical Uses	Smooth muscle relaxant, healing wound in intestine, treatment of dysentery problem, carminative, treatment for skin diseases, prevention and cure pimple, antioxidant, antihistamine, antibacterial, antifungal, wound healing, astringent, antipyretic, treatment of peptic ulcer, gum protective, treatment of insect bite
3.	Production	This essential oil is obtained by steam distillation

4.	Colour/ Appearance	Yellow to yellow-lemon and clear liquid
5.	Odour	Special herby note characteristic, spicy turmeric odour
6.	Specific gravity (20/20 c)	0.9160-0.9366
7.	Refractive index (20c)	1.5023-1.5138
8.	Solubility	Very soluble in ethanol
9.	Stability	Not stable to excess heat, acidic and basic pH. Reactive with reducing and oxidizing agent.
10.	Chemical constituents	Turmerone, zingiberene, phellandrene, sabinene, borneol
11.	Uses	0.5-5.0%
12.	Application	Turmeric oil is added in skin care products, facial wash products, toners and other cosmetics especially cosmetics for sensitive skin.
13.	Storage/ Shelf life	The essential oil has shelf life of 1 year when kept in cool preferably at about 20-25 c dry place and protected from light in their original packaging.

14.	Caution for use	Turmeric oil is contraindication to pregnancy woman. This essential oil may cause skin irritation. This oil must be added at the end of cold preparations and at 35-40°C for emulsions while cooling. The activities of essential oil are best maintained when not heated.

The oil is extracted from the rhizome by steam distillation and the powder is the ground up rhizome. The curcumin contained in turmeric is what gives it such amazing health properties. The Oil has a spicy gingery orange perfume and is slightly green to a yellow color and the powder a bright yellow to a yellow/orange color.Turmeric oil is used in aromatherapy to balance, stimulate and relax. It is anti-inflammatory, anti-arthritis and anti-oxidant oil. It is very strong so should should be used sparingly, 3 drops to a tablespoon of a carrier oil like canola or grapeseed oil. It blends well with ginger or ylang ylang oils.

2.8 COIR MAT

Coir mats are produced from the fibres of coconut. Coir fibres are found between the husk and outer shell of the coconut. The individual fibre cells are narrow and hollow,with thick walls made of cellulose. They are pale when immaturebut later become hardened and yellowed as a layer of lignin is deposited on their walls. There are two types of coir.

Brown fibre

The fibrous husks are soaked in pits or in nets in a slow moving body of water to swell and soften the fibres. The long bristle fibres are separated

known as *wet-milling*. The mattress fibres are sifted to remove dirt and other rubbish, dried in the sun and packed into bales. Some mattress fibre is allowed to retain more moisture so that it retains its elasticity for 'twisted' fibre production. The coir fibre is elastic enough to twist without breaking and it holds a curl as though permanently waved. Twisting is done by simply making a rope of the hank of fibre and twisting it using a machine or by hand. The longer bristle fibre is washed in clean water and then dried before being tied into bundles or hunks. It may then be cleaned and 'hackled' by steel combs to straighten the fibres and remove any shorter fibre pieces. Coir bristle fibre can also be bleached and dyed to obtain hanks of different colours.

White fibre

The immature husks are suspended in a river or water-filled pit for up to ten months. During this time micro-organisms break down the plant tissues surrounding the fibres to loosen them — a process known as retting. Segments of the husk are then beaten by hand to separate out the long fibres which are subsequently dried and cleaned. Cleaned fibre is ready for spinning into yarn using a simple one-handed system or a spinning wheel. Brown coir is harvested from fully ripened coconuts. It is thick, strong and has high abrasion resistance. It is typically used in mats, bushes and sacking. Mature brown coir fibres contain more lignin and less cellulose than fibres such as flax they are ripe. White coir fibres are harvested from the coconuts before they ripe. These fibres are white or light brown in colour and are smoother and finer, but also weaker. They are generally spun to make yarn that is used in mats or rope.The coir mats have high water absorption capacity. The coir have been used as a substrate for growing vegetable since less amount of water is enough for the growth.

Total world coir fibre production is 250,000 tonnes. The coir fibre industry is particularly important in some areas of the developing world. India, mainly the coastal region of Kerala State, produces 60% of the total world supply of white coir fibre. Sri Lanka produces 36% of the total world brown fibre output. Over 50% of the coir fibre produced annually throughout the world is consumed in the countries of origin, mainly India. Together India and Sri Lanka produce 90% of the 250,000 metric tons of coir produced every year.

OBJECTIVES

3. OBJECTIVES

1. To study the effect of different physical agents (UV-irradiation and autoclavable covers) on sanitary napkin
2. To test the antimicrobial activities of leaf extracts from neem plant (*Azadirachta indica*) and turmeric (*Curcuma longa*)
3. To optimize the herbal extract composition (temperature, dilution ratio and time of extraction) using orthogonal analysis.
4. To test the herbal treated sanitary napkins against specific skin diseases causing microorganisms (*Microsporum sp., Trichophyton sp., Klebsiella sp., Staphylococcus aureus., Proteus vulgaris*).
5. To study the blood holding capacity of modified herbal sanitary napkin and conventional napkins using goat blood.
6. To increase the blood holding capacity of modified herbal sanitary napkin by using coir mat
7. To investigate the antimicrobial property of modified herbal sanitary napkin.
8. To study the antimicrobial property of modified herbal sanitary napkin for different time intervals(up to 10 days)

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1 ASSESSING THE EFFECT OF PHYSICAL AGENTS ON SANITARY NAPKINS

Sterilization is a process that destroys or removes all viable microorganisms, including viruses. Any material that has been subjected to this process is said to be sterile. A sterile object is totally free of viable microorganisms, spores, and other infectious agents. These terms should be used only in the strictest sense for methods that have been proved to sterilize. An object cannot be slightly sterile or almost sterile—it is either sterile or not sterile.

Heat and other physical agents are normally used to control microbial growth and sterilize objects, as can be seen from the continual operation of the autoclave in every microbiology laboratory. The four most frequently employed physical agents are heat, low temperatures, filtration and radiation.

4.1.1 ULTRAVIOLET IRRADIATION

4.1.1.1 Principle

Ultraviolet radiation ranges in wavelength from approximately 100 nm to 400 nm. It is most lethal from 240 nm to 280 nm (with a peak at 260 nm). In everyday practice, the source of UV radiation is the germicidal lamp, which generates radiation at 254 nm. Owing to its lower energy state, UV radiation is not as penetrating as ionizing radiation. Because UV radiation passes readily through air, slightly through liquids, and only poorly through solids, the object to be disinfected must be directly exposed to it for full effect.

As UV radiation passes through a cell, it is initially absorbed by DNA. Specific molecular damage occurs on the pyrimidine bases (thymine and cytosine), which form abnormal linkages with each other called *pyrimidine dimers*. These bonds occur between adjacent bases on the same DNA strand and interfere with normal DNA replication and transcription. The results are inhibition of growth and cellular death. In addition to altering DNA, UV radiation also disrupts cells by generating toxic photochemical products (free radicals). Ultraviolet rays are a powerful tool for destroying fungal cells and spores, bacterial vegetative cells, protozoa, and viruses. Bacterial spores are about 10 times more resistant to radiation than are vegetative cells, but they can be killed by increasing the time of exposure.

One major disadvantage of UV is its poor powers of penetration through solid materials such as glass, metal, cloth, plastic, and even paper. Another drawback to UV is the damaging effect of overexposure on human tissues, including sunburn, retinal damage, cancer, and skin wrinkles.

4.1.1.2 Procedure

4.1.1.2.1 Effect of variations in distance from the UV lamp upon the microorganisms

The sanitary napkins were placed in the Laminar Flow Chamber on a sterile stand and exposed to ultraviolet irradiation from the germicidal lamp for a period of 60 minutes. The distance from the UV lamp was varied from 5 cm to 30 cm. The enumeration of microorganisms on each of the sanitary napkin samples was carried out. The results were tabulated.

4.1.1.2.2 Enumeration of microorganisms

After sterilization, the number of microorganisms remaining in the

small piece (10cm X 1cm) was cut aseptically using sterile scissors. It was then aseptically transferred, using sterile forceps, to a conical flask containing 100 ml of sterile nutrient media. Then the conical flask was incubated in a shaker spinning at 120 rpm for overnight.

The number of microorganisms was rapidly enumerated by turbidimetric methods. The microbial cells scatter light striking them. Because microbial cells in a population are of roughly constant size, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number. When the concentration of bacteria reaches about 10 million cells (10^7) per ml, the medium appears slightly cloudy or turbid. Further increases in concentration result in greater turbidity and less light is transmitted through the medium. The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to bacterial concentration at low absorbance levels. Thus population growth can be easily measured spectrophotometrically as long as the population is high enough to give detectable turbidity.

About 2 ml of the culture was aseptically transferred into a clean glass cuvette. The spectrophotometer was calibrated using sterile nutrient media as blank. The absorbance of the culture was measured at 650 nm and the optical density readings were tabulated.

4.1.1.2.3 Effect of variations in time of UV irradiation upon the microorganisms

The sanitary napkins were placed in the Laminar Flow Chamber on a sterile stand and exposed to ultraviolet irradiation from the germicidal lamp at a distance of 5 cm away from the lamp. The times of exposure to UV irradiation was varied from 60 to 420 minutes. The enumeration of

microorganisms on each of the sanitary napkin samples was carried out as in section 4.1.1.2.2. The results were tabulated.

4.1.2 STERILIZATION USING AUTOCLAVE

4.1.2.1 PROCEDURE

The sanitary napkins were carefully packed in sealed autoclavable covers and placed in pressure cookers. Sterilization process was carried out at 121 °C, 15 psi for 20 minutes. The samples were taken from the pressure cooker aseptically. The number of microorganisms was enumerated. The enumeration of microorganisms was carried out according to the procedure outlined in section 4.1.1.2.2.

4.2 EFFECT OF HERBAL AGENTS ON SANITARY NAPKINS

4.2.1 ASSESSING THE ANTIMICROBIAL EFFECT OF HERBAL EXTRACTS

4.2.1.1 Gel diffusion tests

If a rapidly growing aerobic or facultative pathogen like *Staphylococcus is* being tested, a disk or gel diffusion technique may be used to save time and media. The principle behind the assay technique is fairly simple. When an antibiotic-impregnated disk is placed on agar or a well is punched in the gel and filled with herbal extract on agar previously inoculated with the test bacterium, it picks up moisture and the antimicrobial compound diffuses radially outward through the agar, producing an antimicrobial compound concentration gradient. The antimicrobial compound is present at

susceptible microorganisms (resistant organisms will grow up to the disk/ well). As the distance from the disk / well increases, the antimicrobial compound concentration drops and only more susceptible pathogens are harmed. A clear zone or ring is present around an antimicrobial compound disk or well after incubation if the agent inhibits bacterial growth. The wider the zone surrounding a disk or well, the more susceptible the pathogen is. Zone width also is a function of the antimicrobial compound's initial concentration, its solubility, and its diffusion rate through agar. Thus zone width cannot be used to compare directly the effectiveness of two different antimicrobial compounds. Currently the disk diffusion test most often used is the Kirby-Bauer method, which was developed in the early 1960s at the University of Washington Medical School by William Kirby, A.W. Bauer, and their colleagues. An inoculating loop or needle is touched to four or five isolated colonies of the pathogen growing on agar and then used to inoculate a tube of culture broth. The culture is incubated for a few hours at 35°C until it becomes slightly turbid and is diluted to match a turbidity standard. A sterile cotton swab is dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of a Nutrient agar plate. After the agar surface has dried for about 5 minutes, the appropriate antimicrobial compound disks are placed on it, either with sterilized forceps or with a multiple applicator device. The plate is immediately placed in a 35°C incubator. After 16 to 18 hours of incubation, the diameters of the zones of inhibition are measured to the nearest mm. Kirby-Bauer test results are interpreted using a table that relates zone diameter to the degree of microbial resistance.

4.2.1.2 Broth dilution tests

Dilution susceptibility tests can be used to determine MIC and MLC

concentrations in the antimicrobial extract is prepared and inoculated with standard numbers of the test organism. The lowest concentration of the antimicrobial compound resulting in no growth after 16 to 20 hours of incubation is the MIC.MIC is also defined as the minimum concentrarion required to kill 50% of microbial population. The MLC can be ascertained if the tubes showing no growth are subcultured into fresh medium lacking the antimicrobial compound. The lowest antimicrobial compound concentration from which the microorganisms do not recover and grow when transferred to fresh medium is the MLC. The agar dilution test is very similar to the broth dilution test. Plates containing nutrient agar and various amounts of antimicrobial compound are inoculated and examined for growth. Recently several automated systems for susceptibility testing and MIC determination with broth or agar cultures have been developed.

4.2.1.3 Procedure:

4.2.1.3.1 Collection of plant materials

The leaves of the following plants were used in this study:

1. *Azadirachta indica*
2. *Curcuma longa*

The leaves of the plants were collected from various locations after ascertaining the exact identity of the plant and confirming the plant species. The leaves were carefully examined and old, insect-damaged, fungus – infected leaves, twigs and flowers were removed. Healthy leaves were spread out and dried in the laboratory under shade at room temperature for 5-8 days or until they broke easily by hand. Once completely dry, leaf material was ground to a fine powder using a mill or electric blender.

4.2.1.3.2 Preparation of herbal extracts

The extracts of the neem and turmeric leaves were prepared as follows: Extract using Petroleum ether is prepared by optimising the condition for extraction using orthogonal analysis. The parameters used are dilution ratio, temperature and time of extraction. Turmeric oil extract was prepared using Tween20 (1:5 dilution)

4.2.1.3.3 Optimisation of herbal extracts preparation using Orthogonal analysis

The conditions for extracting the antimicrobial components from the leaves of the herbs were optimized by orthogonal design of the various parameters of the extraction process and analyzing the results by statistical methods. Effects of single factors such as temperature, time, and dilution ratio were initially investigated.

On this basis, employing the orthogonal design, the optimum extraction conditions for antimicrobial components from the various herbs were determined by Agar diffusion method.

The optimum extracting conditions of antimicrobial phytochemicals from the leaves of the herbs were determined by adopting L9 (3^3) orthogonal experiments.

4.2.1.3.4 Preparation of inoculum

Pure cultures of the following microorganisms: *Klebsiella pneumoniae, Staphylococcus aureus, Proteus vulgaris were* used for this test.

The lyophilized pure cultures were thawed and inoculated on to nutrient broth and incubated overnight. Then, the cultures were swabbed onto sterile nutrient agar in petri plates. The growth and colony morphology of the

subcultured in conical flasks containing sterile nutrient broth. The cultures were incubated in bacteriological shaker-incubators. The nutrient broth cultures were allowed to grow until the optical density reached 1.000 and then they were subcultured further.

4.2.1.3.4 Assessing the antimicrobial activity by Gel diffusion method

Antimicrobial activity of the herbal extracts was checked by agar gel diffusion method. The cultures were grown in nutrient broth and incubated at 37°C, for 24 h. After incubation period is finished the O.D. of the culture was adjusted to 1.0 with sterile nutrient broth. Then 25 ml of sterile molten nutrient agar was poured into sterile petriplate and allowed to solidify. Then 0.1 ml of the microbial culture was inoculated on the agar plates and the culture was uniformly spread using a sterile glass rod (Spread plate technique). The wells were bored with 8mm borer in the agar. Then 100µl of the herbal extract was added in each well. The plates were incubated at 37°C for 24 h. After incubation period was finished the zone of inhibition was measured and recorded (Talaro and Talaro 2002).

4.2.1.3.5 Determination of MIC of herbal extracts (extracted overnight) by broth dilution method

The cultures were freshly subcultured in nutrient broth and incubated in a shaker until the optical density reached 1.000. Then about 9 ml of *S.aureus* culture was aseptically transferred into sterile test-tubes. About 1 ml of herbal extract (concentration varying from 5mg/ml-25 mg/ml) was aseptically added to the culture in the test-tube. The decrease in the optical density was measured spectrophotometrically at 650 nm, after calibrating the spectrophotometer with sterile media mixed with herbal extract as blank.

The experiment was repeated with other herbal extract using all the other microbial cultures as well. Finally, equal volumes of the herbal extracts were mixed together and the mixed extract was prepared. Then the experiment was repeated with the mixed herbal extract using all the microbial cultures. The results of the experiments were tabulated (Prescott *et al* 2002).

4.3 IDENTIFYING THE COMPOUNDS THAT POSSESS ANTIMICROBIAL ACTIVITY BY TLC

4.3.1 TLC

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action. As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluor which fluoresces everywhere *except* where an organic compound is on the plate.)

4.3.2 PROCEDURE

A TLC plate is prepared by coating a thin layer of stationary phase. The stationary phase is usually a mixture of silica and water in the ratio 1:2.The plate is placed at 80°C for drying. The mobile phase is prepared using ethyl acetate, distilled water and ethanol in the ratio1:5:5 and kept in a beaker for 2 hrs for complete saturation. A small spot of herbal extract is applied to the plate, about one centimeter from the base. The plate is then dipped in to the mobile phase and placed in a sealed container. The mobile phase moves up the plate by capillary action and meets the sample mixture, which is dissolved and is carried up the plate by the solvent.

A standard flavanoid is used as control and the presence of flavonoids in the herbal extract is identified by comparing it with the standard.

4.4 PREPARATION OF HERBAL SANITARY NAPKINS

4.4.1 MATERIALS REQUIRED

4.4.1.1 Relax™ commercial sanitary napkins

The 'Relax' brand of commercial sanitary napkins was manufactured by Gandhigram Rural Institute and supplied to us by RuTAG, IITM.

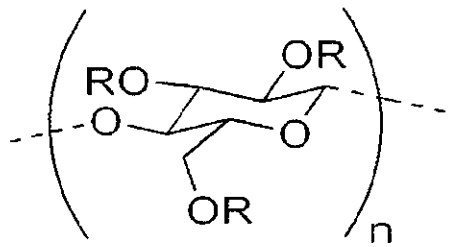
4.4.1.2 Herbal extract

The extracts of the neem and turmeric leaves were prepared as follows:Extract using Petroleum ether is prepared by optimising the condition for extraction using orthogonal analysis.The parameters used are dilution ratio,temperature and time of extraction. Extraction was carried out in an orbital shaker at the optimum temperature for the optimum time period. The plant extracts were filtered. Turmeric oil extract was prepared using Tween20

4.4.1.3 Carboxy methyl cellulose

CMC – sodium salt was used to charge the cloth which is uncharged so that the extract will bind more effectively with the cloth.CMC – sodium salt changes the neutral cloth material into positively charged material by the addition of sodium ion.

Fig. 4.4.1.3.1 Carboxy methyl cellulose



R = H or CH_2CO_2H

4.4.1.4 Dry leaf powder of the herbs

The leaves of the following plants were used in this study:

1. *Azadirachta indica*
2. *Curcuma longa*

The leaves of the plants were collected from various locations after ascertaining the exact identity of the plant and confirming the plant species. The leaves were carefully examined and old, insect-damaged, fungus – infected leaves, twigs and flowers were removed. Healthy leaves were spread out and dried in the laboratory under shade at room temperature for 5-8 days or until they broke easily by hand. Once completely dry, leaf material was ground to a fine powder using a mill or electric blender to a fine powder of about 1.0 mm diameter .The dry leaf powder was thoroughly sieved with a mesh size of 20.

4.4.1.5 Non-woven cotton

Nonwovens are textiles which are neither woven nor knit, such as felt. Non-wovens are typically not strong (unless reinforced by a backing or densified). In recent years, non-woven material has become an alternative to polyurethane foam. Non-woven fabric is typically manufactured by putting small fibers together in the form of a sheet or web, and then binding them either mechanically (as in the case of felt, by interlocking them with serrated needles such that the inter-fiber friction results in a stronger fabric), with an adhesive, or thermally (by applying binder (in the form of powder, paste, or polymer melt) and melting the binder onto the web by increasing temperature). Non-woven fabrics are a manufactured sheet or web of directionally or randomly oriented fibers that are held by adhesion or friction. They do not depend on interlacing of yarn for internal cohesion. They are widely used as they are cheaper and more easily produced than woven cloth. The important types include spun-bond, spun-lace, needle-punched, high loft nonwoven, Spunbond- Meltdown –Spun-bond etc. Pure (100 %) cotton non-woven cloth was purchased.

4.4.2 PROCEDURE

The sanitary napkin typically consists of a porous, diffusible outer layer covering the entire napkin. Inside an absorbent pad of wood pulp, cellulose, absorbent cotton or super absorbent polymer (SAP) is present. Below this, an impermeable layer of polypropylene fabric or plastic film is placed.

In the herbal sanitary napkin, a specially formulated herbal membrane is present. This is prepared as follows:

The non-woven cotton sheet is cut into pieces measuring 15 cm x 10 cm .This sheet is placed in a large glass tray or plate. This sheet is charged by

The sheet was allowed to completely absorb the extract for about an hour. The herbal extract coated cloth sheet is then dried at 70°C for one hour. The pH of the extract coated herbal membrane is checked to be 7, i.e., neutral pH.

The herbal membrane is wrapped around the entire absorbent pad and sealed by stitching the herbal membrane closed. The absorbent pad with the herbal membrane is placed over the impermeable film at the base of the sanitary napkin.

The entire sanitary napkin is covered by the porous, non-absorbent diffusible layer and sealed close by stitching or other methods. A suitable weight is placed on the napkin and is evenly moved over the surface of the sanitary napkin to ensure that the surface is smooth and uniform.

Then an adhesive sticker is pasted at the bottom of the sanitary napkin and the exposed sticky surface is covered by a layer of wax paper. This may be replaced by using a band or belt for holding the napkin in position.

In case of large or extra-large models of sanitary napkins, the size of the herbal membrane used may be increased proportionately.

The sanitary napkin is placed in a suitable plastic cover and sealed. It is then sterilized by autoclaving the package at 120 °C, 15 psi pressure for 20 minutes or by heating in a pressure cooker.

Then the sanitary napkin with the plastic cover is packed in a plastic wrapper and is ready for use (Michael Alary *et al* 2001).

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

5.1 EFFECT OF PHYSICAL AGENTS ON SANITARY NAPKINS

5.1.1 UV IRRADIATION

The effect of various physical agents on sanitary napkins was investigated. The sanitary napkins were subjected to ultraviolet irradiation from a germicidal lamp. The UV irradiation has very limited penetrating power and is useful only for surface sterilization. The studies revealed that UV irradiation from a distance of 5 cm above the sanitary napkin, for a period of at least one hour on both surfaces of the napkin is required (Table and Figure 5.1.1.1 & 5.1.1.2). This obviously did not result in absolute sterility and the number of surviving viable organisms was enumerated. However, this should be sufficient for normal use.

In cases where absolute sterility of the napkins is required, the napkins should be autoclaved with holding period's greater than several hours. If the napkin contains some components like plastic films etc. that are heat-labile, then UV radiation may be employed. However, for most applications, the sanitary napkins may be placed in sealed plastic covers and heated in pressure cookers to achieve significant reduction in the microbial count.

Table 5.1.1.1 Effect of Distance from the UV Lamp on sanitary napkins

S.No	Distance from the UV Lamp (cm)	Optical density at 650 nm
1	2	0.270
2	4	0.389
3	6	0.452
4	8	0.533
5	10	0.579
6	12	0.612

Fig, 5.1.1.1 Effect of Distance from the UV Lamp on sanitary napkins

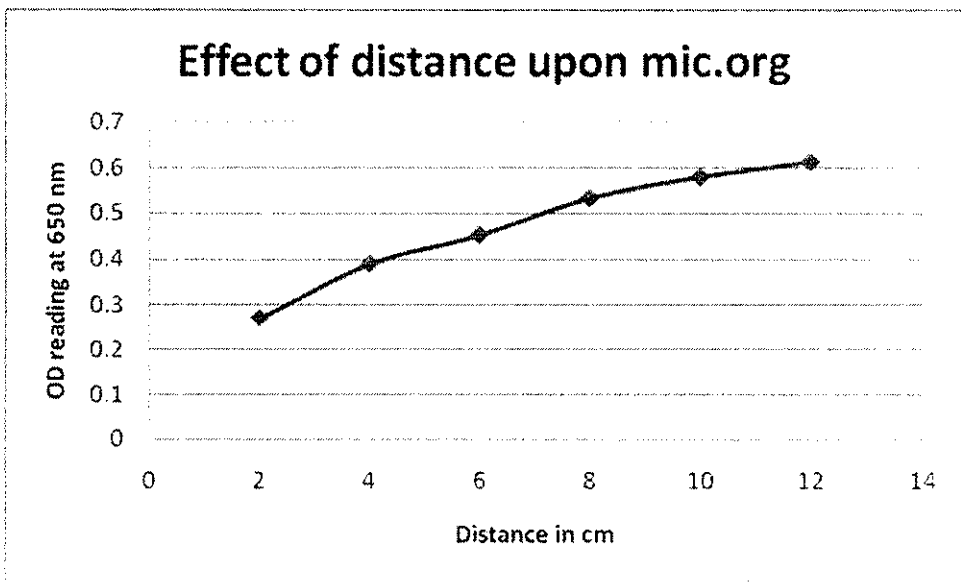
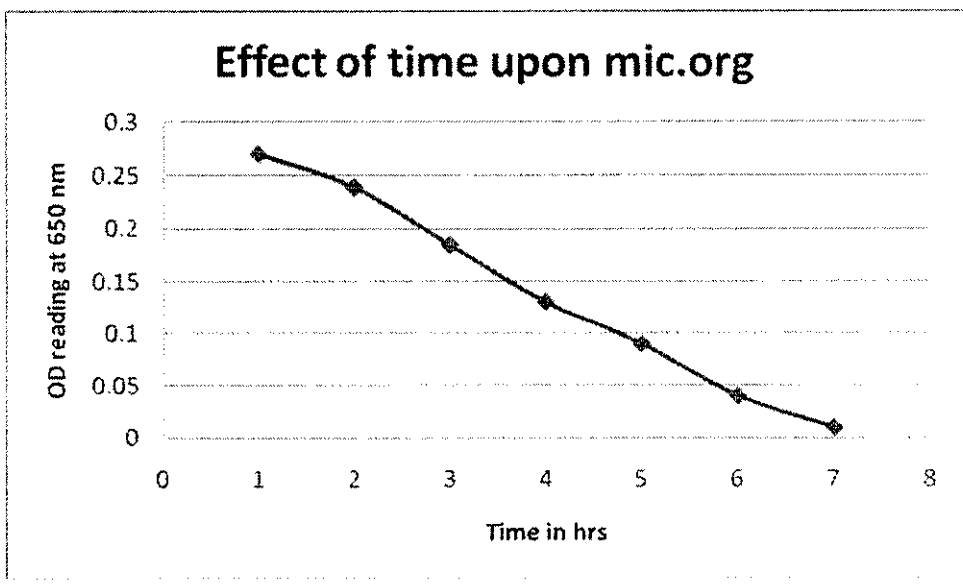


Table 5.1.1.2 Effect of Time of exposure to UV light on sanitary napkins

S.No	Time(hrs)	OD reading at 650 nm
1	1	0.270
2	2	0.239
3	3	0.185
4	4	0.131
5	5	0.090
6	6	0.040
7	7	0.010

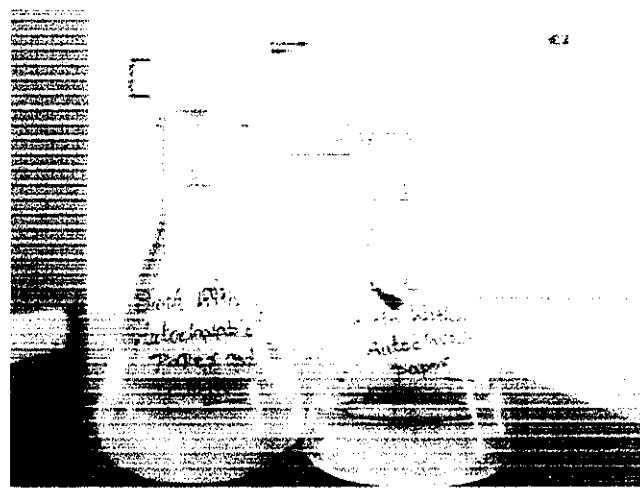
Fig 5.1.1.2 Effect of Time of exposure to UV light on sanitary napkins



5.1.2 STERILIZATION USING AUTOCLAVE

The sanitary napkins were carefully packed in sealed plastic covers and placed in pressure cookers. Sterilization process was carried out at 121 °C, 15 psi for 20 minutes. The samples were taken from the pressure cooker aseptically. The number of microorganisms was enumerated. The enumeration of microorganisms was carried out according to the procedure outlined in section 4.1.1.2.2.

Fig. 5.1.2 Broths inoculated with cloth after autoclave



Broth containing cloth sterilised with autoclavable covers was compared with broth containing sterile broth which is used as control. The broth containing cloth treated with autoclavable covers showed no growth.

5.2 EFFECT OF HERBAL AGENTS ON SANITARY NAPKINS

5.2.1 PRELIMINARY ANTIMICROBIAL STUDIES

The effects of various herbal agents on sanitary napkin were investigated .This was accomplished by incorporating a specially prepared herbal membrane.

The following herbs were chosen from previously published research papers. The aqueous extracts of leaves were prepared by extracting the phytochemicals from the leaves using petroleum ether as a solvent and the antimicrobial effects of the herbs were determined by gel diffusion assay as well as tube dilution tests.

1. *Azadirachta indica*
2. *Curcuma longa*

The herbal extracts were optimised by using orthogonal analysis and the optimum conditions were found by agar diffusion method. The herbal extracts tested (*Azadirachta indica,Curcuma longa*) were found to have significant antibacterial effect and the MIC of herbal extracts were determined by measuring the decrease in optical density readings of the culture at 650 nm.

Moreover, even when filter paper disks wetted with the herbal extracts were placed on the petriplates containing nutrient agar with fully grown microbial colonies, lysis of colonies around the filter plate was observed, giving rise to a zone of clearance around the herbal disks. Even after a period of three days, no microbial colonies were observed in the zone around the herbal disks. This confirmed the microbicidal action of the herbal extracts.

5.2.2 ORTHOGONAL DESIGNING

The conditions for extracting the antimicrobial components from the leaves of the herbs were optimized by orthogonal design of the various parameters of the extraction process and analyzing the results by statistical methods. Effects of single factors such as temperature, time, and dilution ratio were initially investigated.

On this basis, employing the orthogonal design, the optimum extraction conditions for antimicrobial components from the various herbs were determined by Agar diffusion method.

The optimum extracting conditions of antimicrobial phytochemicals from the leaves of the herbs were determined by adopting L9 (3^3) orthogonal experiments.

5.2.2.1 Optimum conditions for extraction of antimicrobial components from *Azadirachta indica*

The herbal extract of neem was prepared by means of orthogonal designing.L9 experiments were carried out and the optimal conditions were found by measuring the zone of inhibition by broth dilution method.

Table 5.2.2.1.1 Optimization of antimicrobial components extracted from *Azadirachta indica* against *Staphylococcus aureus* using orthogonal designing

Initial optical reading of all the cultures ≈ 1.000

Factors and Levels

Level	Temperature (°C)	Extraction Time (min)	Dilution ratio (W:V)
1	55	60	1:10
2	60	120	1:15
3	65	180	1:20

Experiment results and range analysis

Item	A	B	C	OD at 650 nm	Decrease in OD
1	1	1	1	0.600	0.400
2	1	2	2	0.811	0.189
3	1	3	3	0.817	0.183
4	2	1	2	0.753	0.247
5	2	2	3	0.604	0.396
6	2	3	1	0.581	0.419
7	3	1	3	0.598	0.402
8	3	2	1	0.694	0.306
9	3	3	2	0.653	0.347

Table 5.2.2.1.2 Optimization of antimicrobial components extracted from *Azadirachta indica* against *Klebsiella sp.* using orthogonal designing

Factors and Levels

Level	Temperature (°C)	Extraction Time (min)	Dilution ratio (W:V)
1	55	60	1:10
2	60	120	1:15
3	65	180	1:20

Experiment results and range analysis

Item	A	B	C	OD at 650 nm	Decrease in OD
1	1	1	1	0.514	0.486
2	1	2	2	0.718	0.282
3	1	3	3	0.724	0.276
4	2	1	2	0.780	0.280
5	2	2	3	0.511	0.489
6	2	3	1	0.505	0.495
7	3	1	3	0.623	0.377
8	3	2	1	0.601	0.399
9	3	3	2	0.570	0.430

Table 5.2.2.1.3 Optimization of antimicrobial components extracted from *Azadirachta indica* against *Proteus vulgaris* using orthogonal designing

Factors and Levels

Level	Temperature (°C)	Extraction Time (hrs)	Dilution ratio (W:V)
1	55	1	1:10
2	60	2	1:15
3	65	3	1:20

Experiment results and range analysis

Item	A	B	C	OD at 650 nm	Decrease in OD
1	1	1	1	0.556	0.444
2	1	2	2	0.761	0.239
3	1	3	3	0.767	0.233
4	2	1	2	0.770	0.230
5	2	2	3	0.554	0.446
6	2	3	1	0.526	0.474
7	3	1	3	0.548	0.452
8	3	2	1	0.644	0.356
9	3	3	2	0.613	0.387

The optimum conditions for extraction was found to be

Temperature - 60°C

Time of extraction - 3 hrs

Dilution ratio -1:10

5.2.2.2 Optimization of antimicrobial components extracted from *Curcuma longa*

The herbal extract of turmeric was prepared by means of orthogonal designing.L9 experiments were carried out and the optimal conditions were found by broth dilution method.

Table 5.2.2.2.1 Optimization of antimicrobial components extracted from *Curcuma longa* against *Staphylococcus aureus* using orthogonal designing

Inital optical density reading of all the cultures ~ 1.000

Factors and Levels

Level	Temperature (°C)	Extraction Time (min)	Dilution ratio (W:V)
1	55	60	1:10
2	60	120	1:15
3	65	180	1:20

Experiment results and range analysis

Item	A	B	C	OD at 650 nm	Decrease in OD
1	1	1	1	0.400	0.600
2	1	2	2	0.433	0.567
3	1	3	3	0.395	0.605
4	2	1	2	0.388	0.612
5	2	2	3	0.560	0.440
6	2	3	1	0.389	0.611
7	3	1	3	0.372	0.628
8	3	2	1	0.370	0.630
9	3	3	2	0.411	0.589

Table 5.2.2.2.2 Optimization of antimicrobial components extracted from *Curcuma longa* against *Klebsiella sp.* using orthogonal designing

Factors and Levels

Level	Temperature (°C)	Extraction Time (min)	Dilution ratio (W:V)
1	60	60	1:10
2	70	120	1:15
3	80	180	1:20

Experiment results and range analysis

Item	A	B	C	OD at 650 nm	Decrease in OD
1	1	1	1	0.547	0.453
2	1	2	2	0.578	0.422
3	1	3	3	0.540	0.460
4	2	1	2	0.533	0.467
5	2	2	3	0.705	0.295
6	2	3	1	0.534	0.466
7	3	1	3	0.517	0.483
8	3	2	1	0.505	0.495
9	3	3	2	0.556	0.444

Table 5.2.2.2.3 Optimization of antimicrobial components extracted from *Curcuma longa* against *Proteus vulgaris* using orthogonal designing

Factors and Levels

Level	Temperature (°C)	Extraction Time (min)	Dilution ratio (W:V)
1	55	60	1:10
2	60	120	1:15
3	65	180	1:20

Experiment results and range analysis

Item	A	B	C	OD at 650 nm	Decrease in OD
1	1	1	1	0.478	0.522
2	1	2	2	0.519	0.481
3	1	3	3	0.481	0.519
4	2	1	2	0.474	0.526
5	2	2	3	0.646	0.354
6	2	3	1	0.475	0.525
7	3	1	3	0.458	0.542
8	3	2	1	0.450	0.550
9	3	3	2	0.500	0.500

The optimum conditions for extraction was found to be

Temperature - 65°C

Time of extraction - 2 hrs

Dilution ratio - 1:10

5.2.3 DETERMINATION OF MIC

In the broth dilution test, a series of broth tubes containing suitable concentrations in the antimicrobial extract is prepared and inoculated with standard numbers of the test organism. The lowest concentration of the antimicrobial compound resulting in no growth after 16 to 20 hours of incubation is the MIC. MIC is also defined as the minimum concentrarion required to kill 50% of microbial population. The agar dilution test is very similar to the broth dilution test. Plates containing nutrient agar and various amounts of antimicrobial compound are inoculated and examined for growth.

5.2.3.1 Determination of MIC for neem extract against microorganisms

Table 5.2.3.1.1 Determination of MIC of neem extract against *Staphylococcus aureus* by broth dilution method

Time (min)	concentration				
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25mg/ml
30	0.966	0.930	0.921	0.911	0.905
60	0.892	0.860	0.810	0.801	0.8
90	0.818	0.798	0.709	0.710	0.710
120	0.750	0.735	0.612	0.614	0.606
150	0.669	0.658	0.529	0.522	0.515

MIC - 15 mg/ml

Table 5.2.3.1.2 Determination of MIC of neem extract against *Klebsiella sp.* by broth dilution method

Time(min)	Concentration				
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml
30	0.856	0.842	0.820	0.821	0.809
60	0.802	0.788	0.723	0.719	0.720
90	0.734	0.725	0.639	0.638	0.622
120	0.698	0.660	0.551	0.545	0.530
150	0.644	0.587	0.469	0.465	0.452

MIC – 15 mg/ml

Table 5.2.3.1.3 Determination of MIC of neem extract against *Proteus vulgaris* by broth dilution method

Time (min)	Concentration				
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml
30	0.931	0.902	0.897	0.860	0.855
60	0.884	0.834	0.811	0.756	0.759
90	0.821	0.765	0.740	0.668	0.660
120	0.780	0.687	0.644	0.571	0.555
150	0.725	0.601	0.595	0.495	0.478

MIC – 20 mg/ml

Table 5.2.3.1.4 Anti bacterial effect of *Azadirachta indica* in terms of mean diameter zone of inhibition (mm)

Organism	5 mg/ml of extract	10 mg/ml of extract	15 mg/ml of extract	20 mg/ml of extract	25 mg/ml of extract	MIC (mg/ml)
Staphylococcus aureus	11	14	21.5	21	23	15
Klebsiella sp.	11.6	13.3	19	20.5	20.8	15
Proteus vulgaris	0	13.5	13.5	19.5	18.5	20

Fig 5.2.3.1.4.1 Antibacterial effect of neem against *S.aureus* by disc diffusion method

Fig 5.2.3.1.4.2 Antibacterial effect of neem against P.vulgaris

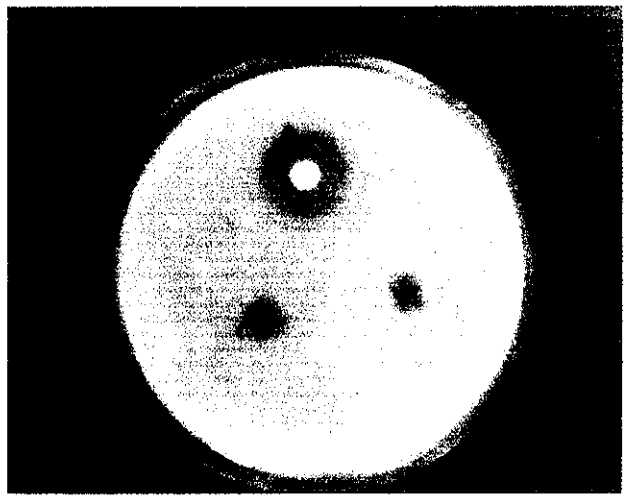


Fig 5.2.3.1.4.3 Antibacterial effect of neem against Klebsiella sp.

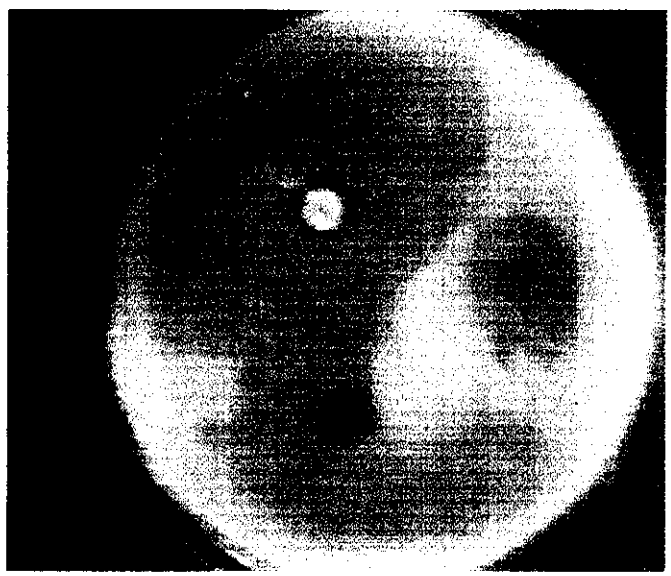


Table 5.2.3.1.5 Anti fungal effect of *Azadirachta indica* in terms of mean diameter zone of inhibition (mm)

Organism	5 mg/ml of extract	10 mg/ml of extract	15 mg/ml of extract	20 mg/ml of extract	25 mg/ml of extract	MIC (mg/ml)
Candida albicans	0	12.5	15	23	24.5	20
Trichophyton sp.	0	10.2	13.5	16.5	22	25
Microsporum sp.,	0	12	14.5	20.8	21.2	20

5.2.3.2 Determination of MIC for turmeric extract against microorganisms

Table 5.2.3.2.1 Determination of MIC of Turmeric extract against *Staphylococcus aureus* by broth dilution method

Time (min)	Concentration				
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25mg/ml
30	0.971	0.955	0.931	0.910	0.905
60	0.905	0.893	0.850	0.820	0.801
90	0.845	0.820	0.789	0.733	0.719
120	0.798	0.779	0.722	0.638	0.636
150	0.720	0.718	0.669	0.510	0.511

MIC – 20 mg/ml

Table 5.2.3.2.2 Determination of MIC of Turmeric extract against *Klebsiella*

Time(min)	Concentration				
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml
30	0.938	0.931	0.928	0.912	0.909
60	0.873	0.868	0.825	0.845	0.810
90	0.791	0.774	0.754	0.761	0.722
120	0.714	0.697	0.678	0.689	0.610
150	0. 658	0.655	0.623	0.598	0.502

MIC – 25 mg/ml

Table 5.2.3.2.3 Determination of MIC of Turmeric extract against *Proteus vulgaris* by broth dilution method

Time (min)	Concentration				
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml
30	0.965	0.942	0.912	0.896	0.885
60	0.912	0.894	0.861	0.828	0.801
90	0.873	0.839	0.820	0.770	0.733
120	0.807	0.771	0.766	0.711	0.628
150	0.765	0.710	0.699	0.656	0.511

MIC – 25 mg/ml

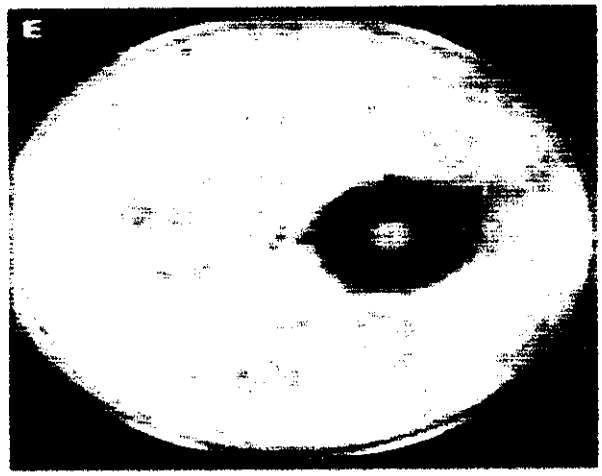
Table 5.2.3.2.4 Anti bacterial effect of *Curcuma longa* in terms of mean diameter zone of inhibition (mm)

Organism	5 mg/ml of extract	10 mg/ml of extract	15 mg/ml of extract	20 mg/ml of extract	25 mg/ml of extract	MIC (mg/ml)
Staphylococcus aureus	12.4	14	15.5	21.2	23	20
Klebsiella sp.	0	13.3	13.5	15.5	22	25
Proteus vulgaris	0	13.5	15.5	15.3	19.5	25

Table 5.2.3.2.5 Anti fungal effect of *Curcuma longa* in terms of mean diameter zone of inhibition (mm)

Organism	5 mg/ml of extract	10 mg/ml of extract	15 mg/ml of extract	20 mg/ml of extract	25 mg/ml of extract	MIC (mg/ml)
Candida albicans	11	11.5	18.5	19.6	19.5	15
Trichophyton sp.	10.5	10.2	22	21.8	23	15
Microsporum sp.,	13	15	21.5	21.5	21	15

Fig 5.2.3.2.1 Anti fungal effect of Turmeric by disc diffusion method



5.3 IDENTIFYING THE COMPOUNDS THAT POSSESS ANTIMICROBIAL ACTIVITY BY TLC

A TLC plate is prepared by coating a thin layer of stationary phase.The stationary phase is usually a mixture of silica and water in the ratio 1:2.The plate is placed at 80°C for drying.The mobile phase is prepared using distilled water,ethyl acetate and ethanol in the ratio 5:1:5 and kept in a beaker for 2 hrs for complete saturation. A small spot of herbal extract is applied to the plate, about one centimeter from the base. The plate is then dipped in to the mobile phase and placed in a sealed container. The mobile phase moves up the plate by capillary action and meets the sample mixture, which is dissolved and is carried up the plate by the solvent.

A standard flavanoid is used as control and the presence of flavonoids in the herbal extract is identified by comparing it with the standard.

Fig. 5.3.1 TLC for neem extract

Fig 5.3.2 TLC for turmeric extract

5.4 PREPARATION OF HERBAL TREATED SANITARY NAPKINS

In the herbal sanitary napkin, a specially formulated herbal membrane is present. This is prepared as follows:

Two non-woven cotton sheets were cut into pieces measuring 15 cm x 10 cm .One sheet is placed in a large glass tray or plate.This sheet is charged by adding 1% CMC. And the other sheet is kept uncharged. To both the sheet, about 15 ml of extract was carefully added. The sheets were allowed to completely absorb the extract for about an hour. The herbal extract coated cloth sheets were then dried at 70°C for one hour. The pH of the extract coated herbal membrane is checked to be 7, i.e., neutral pH. The optical density reading for the herbal treated cloth (charged and uncharged) and untreated cloth was measured and compared.

Table 5.4.1 Comparison of optical density of treated (charged and uncharged) and untreated cloth

TYPE	OD READING AT 650 nm
Control	0
Cloth untreated	0.79
Cloth treated(CHARGED)	0.31
Cloth treated(UNCHARGED)	0.39

- Cloth that is treated with herbal extract shows 51% decrease in micro organism than the cloth that is untreated.

- Cloth that is treated and charged shows 20% decrease in microorganism growth than the cloth that is treated with herbal extract but uncharged.

The antimicrobial activity of charged and uncharged cloth was checked by agar diffusion method. The cultures were grown in nutrient broth and incubated at 37°C, for 24 h. After incubation period is finished the O.D. of the culture was adjusted to 1.0 with sterile nutrient broth. Then 25 ml of sterile molten nutrient agar was poured into sterile petri plate and allowed to solidify. Then 0.1 ml of the microbial culture was inoculated on the agar plates and the culture was uniformly spread using a sterile glass rod (Spread plate technique). Discs of charged and uncharged cloth are prepared. The discs are placed in the petriplate. The plates were incubated at 37°C for 24 h. After incubation period was finished the zone of inhibition was measured and recorded.

5.5 CHECKING THE ANTIMICROBIAL PROPERTY OF MODIFIED HERBAL SANITARY NAPKIN FOR DIFFERENT TIME INTERVALS

The herbal extract is prepared as follows:

The non-woven cotton sheets were cut into pieces measuring 15 cm x 10 cm .The sheets were placed in a large glass tray or plate. This sheet is charged by adding 1% CMC. To this sheet, about 15 ml of extract was carefully added. The sheet was allowed to completely absorb the extract for about an hour. The herbal extract coated cloth sheet is then dried at 70°C for one hour. The herbal treated cloths were kept for different time intervals and the antimicrobial activity of the treated cloths was determined by measuring the optical density reading at 650 nm.

The optical density reading showed that the herbal treated cloth was more effective against microorganisms for a period of 10 days. The herbal treated cloth that was kept for 10 days showed 40% reduction in the number of microorganisms when compared with the untreated cloth.

Table 5.5.1 Optical density reading of the herbal treated sanitary napkin that was kept for different period of time

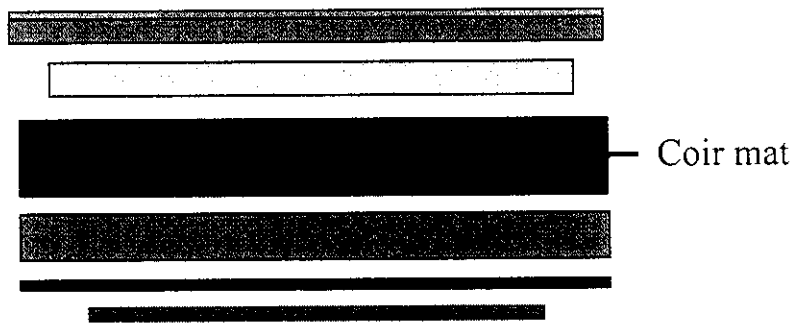
TIME INTERVAL(hrs)	OD READING AT 650 nm
Untreated	0.79
0	0.39
24	0.39
48	0.40
72	0.42
168	0.45
240	0.47

5.6 PREPARATION OF MODIFIED SANITARY NAPKIN

The sanitary napkins consist of a top sheet, a diffusion layer, an absorption layer, a back panel, and adhesive tape to prevent shifting. The role of the diffusion layer is to quickly accept the bodily fluid from the top sheet and transfer it to the absorption layer. There are basically two types of absorption layers---either a laminate in which a superabsorbent polymer is sandwiched by a tissue or nonwoven cloth, or a mixture of a superabsorbent polymer and pulp wrapped by a tissue around a nonwoven cloth. Usually

wood pulp is used as the absorption layer in conventional napkins. In this experiment the absorption layer was modified by the addition of coir mat to increase the absorption capacitynof the napkins. A coir mat is placed in between the two layers of wood pulp and the blood absorbing capacity of the modified napkin was found out.

Fig. 5.6.1 Modified herbal treated sanitary napkin



- Green = Herbal Membranes (Totally 2)
- Orange = Electrostatic surface
- Yellow = Diffusion layer
- Brown = Absorption layer
- Black = Back panel
- Pink = Adhesive tape

5.7. BLOOD ABSORBING CAPACITY OF THE SANITARY NAPKINS

The blood absorbing capacity of the sanitary napkins have been assessed as per the method given in the ISI: 5405:1980, called the Indian Standard Specification for Sanitary Napkins. The sanitary napkin shall absorb

When a standard weight of 1 kgf is placed for 1 minute on the portion where the fluid was absorbed, the fluid shall not leak through to the bottom or the sides of the napkin.

The herbal napkin absorbed 30 ml of blood without leakage even after the application of pressure. Hence, our herbal napkins conform to the ISI standards. Moreover, the total blood absorbing capacity of the modified herbal napkin was found to be 72 ml.

Table 5.7.1 Comparison of blood absorbing capacity of modified sanitary napkin with conventional sanitary napkins

S.No	Sanitary Napkin	Whether napkin absorbed 30 ml of blood without leakage	Total volume of blood absorbed (ml)	Any signs of leakage
1	Herbal	Yes	72	No
2	Relax	Yes	32	Yes
3	Whisper	Yes	42	No
4	Kotex	Yes	41	No
5	Stayfree	Yes	40	No

CONCLUSION

6. CONCLUSION

- In this project, the effect of physical and herbal agents on sanitary napkins was studied in order to develop a technology to manufacture new type of innovative, cost effective and eco friendly herbal sanitary napkins.

- Effects of various physical agents like UV irradiation and Autoclavable covers on sanitary napkins were studied in different experiments. Our work revealed that for most applications, the sanitary napkins may be placed in sealed autoclavable covers and heated in pressure cookers to achieve significant reduction in the microbial count.
- The effects of dry leaves of two different medicinal plants on sanitary napkins were investigated. The Petroleum ether extract of the leaves and dried leaf powders had appreciable antimicrobial activity. The conditions for extracting the antimicrobial components from the leaves of the herbs were optimized by orthogonal designing and from the analyses it was observed that the variations in responses between different microbial cultures when treated with the leaf extract of the same plant are very small. However, there are significant variations in the responses of the microbial cultures to the extracts from different plants.

- The herbal sanitary napkins were manufactured by adding a specially formulated herbal membrane and dry leaf powders to the existing Relax^{TM} sanitary napkins. The durability of modified sanitary napkin was monitored for about 10 days and the result

showed that the treated napkin was found effective over a considerable period of time.

- The blood absorbing capacity of sanitary napkin was increased by adding a layer of coir mat in between two layers of wood pulp. The blood absorption capacity was doubled when compared with the ordinary napkin.

- The herbal sanitary napkins are very economical is sure to improve the quality of life of people who use it. Hence the herbal sanitary napkins are cost effective and produce optimum results for the expenditure. The proven efficacy of the sanitary napkins ensures that the customers get good value for their money. Thus this project has managed to prepare an innovative product that is ready for market-use.

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