CHAPTER - 7
SUMMARY
AND
CONCLUSION
Summary

On the earth, plants occupy a unique position since they are the foundation for life. They are the main producers in all food chains. The plants directly supply 90% of human calorific intake and about 80% of protein intake.

The temperate, tropical and subtropical species were included in Asiatic flora, which provide systems for plants or plant parts based drugs and these systems play a keystone part in the healthcare systems of many countries in Asia. Asia’s 80% population are still depending on traditional and folk remedies for day-to-day medical needs.

Human foodstuff decisions and consuming examples have been determined by impulse and money related status and have been partial by the advancement of foodstuffs all the more on their expense, taste or comfort, and less for their sustenance. In any case designs related with a lower hazard of ceaseless sicknesses, including normal structures of growth.

One of the first written records concerning curative drugs and narcotic substances was found on a clay tablet in Assyrian cuneiform script dating back to 2,700 BC. Ayurveda has well-known treaties known as Charak Samhita and Susrut Samhita, the oldest and very first written document of Ayurveda (900 BC). It describes 341 herbal products as medicine and more importantly classify these in terms of physiological activity.

The traditional medicine used in India known popularly as the Indian arrangement of prescription incorporates Siddha, Ayurveda, Unani and Naturopathy. Natural products (crude drug extracts and pure compounds) obtained for either marine or terrestrial origin.

Starting from 1800 A.D., the isolation and characterization of herbal extracts became a major part of pharmacopoeias. Around 15-20 thousand plants are known to have great restorative properties of which just around 7,000 – 7,500 are, no doubt utilized by conventional therapeutic professionals. The Ayurvedic system utilizes 700 plant species, Siddha 650, Unani 750 and modern medicine (Allopathy) about 30 plant species.
Herbal technology is the biggest revenue earner in India after information technology. Plant-based drugs or drug products are continuously progressively favored in medicinal science. WHO has stressed on the need to guarantee the quality control of herbs and home grown plans by utilizing current techniques.

BHP contains 233 monographs and quality control tests, and Chinese Herbal Pharmacopoeia contain 1751 monographs of substances and articles, BHC contains 84 monographs of medicinal plants. German Commission E has 330 monographs for drugs used in German folk medicine.

In contrast, most-chronic pain states are related with irregularities of the normal physiological conduit, giving rise to an improved amount of pain related with a mild harmful stimulus (hyalgaliesia), pain evoked by a non-toxic stimulus (allodynia), or impulsive spasms of pain with no precipitating stimulus. Parts determined from characteristic items have used structure the starting involving ache treatment.

Scholastic examination has kept on being a solid pioneer in the field of common items, particularly concerning recently found concoction elements. Torment administration and medication compulsion scrutinize now initially centered around common mixes solely. The pharmacology of torment has end up being a complex field, and as more frameworks methodologies were investigated, more potential medication targets are continuously distinguished. The reactions of as of now utilized torment meds shift focused around the classification of executor utilized.

Temperature of body goes between 37.22°C and 40.57°C onward is known as pyrexia while rise of body temperature above 41.66°C is the hyperpyrexia. Imbalance of heat regulating mechanism responsible for the rise of body temperature. Toxins (pyrogens) act on WBC and produce endogenous pyrogen. It acts directly on anterior hypothalamus and the body temperature was elevated. Fever occurs due to any of the causes such as infections (e.g. pneumonia, typhoid, fever, etc.), injury to nervous centres, dehydration, tissue destruction, administration of some drugs, etc.
Chemical addiction is often extremely difficult and painful to master. One common management comprises administration of opioids and opioid analgesics in reducing doses over an extended period. For example, methadone is well-recognized for treating heroin addiction by administering in gradually diminishing amounts. While such regimens do tend to ease several of the withdrawal indications related with decontamination, they take months to complete it and are therefore slightly successful in helping the addict take a permanent step away from chemical addiction.

Inflammatory diseases in the human beings are coming forth as important health issue of the 21st C. Inflammatory ailments and its complications like gastrointestinal symptoms including diarrhea, abdominal pain, GI hemorrhage, anemia, weight loss, spondylitis arthritis, ankylosing, uveitis, iritis sclerosing cholangitis, pyoderma gangrenosum and erythema nodosum take major on the caliber of life elderly and the health care costs of the club.

Therefore, it is envisaged that in order to subside the complications and toxic effects of allopathic medicines; traditional herbal medicines should be worked in a suitable delivery dosage forms as they are thought to be secure because of natural descent.

The present work relates usually to a composition and method for managing pain and inflammation. More principally, the present development relates to the pain and inflammation managing composition, which contains extracts from plant species. The present invention provides a conventional and novel composition which transdermally administered plant extracts for ameliorating ache and swelling.

In the existing work, extracts of coarsely powdered shade dried plant materials i.e. fruit of Lagenaria siceraria and leaves of Ocimum gratissimum and Moringa oleifera were selected for the development of polyherbal formulations. The plant parts were subjected to assessment of quality control factors for crude material and then subjected to extraction with different solvent system.
The concentrated extracts were subjected to preliminary physical and phytochemical investigation in demand to evaluate the quality of plant parts and understand the nature of active constituents present. The aqueous and methanolic extracts of all three plants were used for the screening of swelling suppression, analgesic and fever reducing potential. Further the aqueous extracts of *Lagenaria siceraria* and *Moringa oleifera* and methanolic extract of *Ocimum gratissimum* are used for the development of conventional semisolid formulations and transdermal patches.

*Lagenaria siceraria, Ocimum gratissimum* and *Moringa oleifera* were morphologically and microscopically evaluated for the conformation of their identity. The *Lagenaria siceraria* are greenish with distinguishing odor and slightly acrimonious in sense of taste. The fruit is dumb bell in shape. The leaves of *Ocimum gratissimum* are dark green with aromatic smell and acrimonious in taste. The leaves are simple, opposite, decussate, lanceolate to oblong in shape. The leaves of *Moringa oleifera* are greenish with distinctivesmell and acrid in taste. The leaves are ovate, pinnate in shape.

In the microscopic study, *Lagenaria siceraria* shows presence of anisocytic stomata, vascular bundles fenced by 3-4 sclerenchymalayer and trichomes of covering and collapsed type are present. Transverse section of *Ocimum gratissimum* leaf shows xylem vessels, phloem fibers and Multi cellular, collapsed, uniseriate covering trichome. Transverse section of *Moringa oleifera* leaf shows spiral vessels, trichomes, anomocytic stomata and rosette calcium oxalate crystals.

Shade dried powdered fruit of *Lagenaria siceraria*, leaves of *Ocimum gratissimum* and leaves of *Moringa oleifera* were evaluated for phytochemical parameter loss on drying, extractive value and ash value. The total ash value, acid insoluble ash value and water soluble ash value of *Lagenaria siceraria* was discovered to be 85.90 % w/w, 0.89 % w/w and 4.2 % w/w correspondingly. The total ash value, acid insoluble ash value and water soluble ash value of *Ocimum gratissimum* was discovered to be 11.2 % w/w, 1.2 % w/w and 11.9 % w/w correspondingly. The total ash value, acid insoluble ash value and water soluble ash value of *Moringa oleifera* was discovered to be 19.56 % w/w, 2.90 % w/w and 4.80 % w/w.
The extractive values of *Lagenaria siceraria* were discovered to be 5.20 % w/w and 6.26 % w/w correspondingly. The extractive values of *Ocimum gratissimum* were found to be 29.5 % w/w and 16.6 % w/w correspondingly. The extractive values of *Moringa oleifera* were found to be 29.60 % w/w and 13.8 % w/w correspondingly. The loss on drying of *Lagenaria siceraria*, *Ocimum gratissimum* and *Moringa oleifera* were established to be 4.54 % w/w, 15.5 % w/w and 14.5 % w/w correspondingly. The higher amount of ash in all the three plant materials indicates presence of inorganic salt and metals in all the fruits.

All three plant materials were used for estimation of tannins and total phenolic in which the *Moringa oleifera* found with the highest amount of total tannins (22.30 %). The phenolic content in *Moringa oleifera* was found to be 60.21 % w/w. *Ocimum gratissimum* contains 7.23 % tannin and 65.91 w/w of phenolic content, while tannins and phenolic content of *Lagenaria siceraria* were to be 3.12 % and 50.24 % w/w respectively.

All three plant materials were extracted by two solvents viz. methanol and water. The % yield of aqueous and methanolic extract of *Lagenaria siceraria*, *Ocimum gratissimum* and *Moringa oleifera* was 9.12 % w/w and 11.0 % w/w, 12.15 % w/w and 7.55 % w/w, 7.55 % w/w and 9.1 % w/w respectively.

The methanol and aqueous extracts of all three plants were examined for the phytoconstituents. Methanolic extract of *Lagenaria siceraria* showed the presence of the tannin, phenolic compound, flavonoids, saponins, carbohydrates and proteins, while its watery extract exposed the presences of carbohydrates, flavonoids, tannins, proteins and vitamins. Methanolic *Ocimum gratissimum* showed the existence of tannins, steroids, saponins, flavonoids, terpenoids, and phenolic compounds, while its aqueous extract disclosed the presences of saponins, glycosides and flavonoids. Methanolic extract and aqueous concentrate of *Moringa oleifera* indicated the existence of flavonoids, tannins, glycoside, alkaloids, saponins, steroids, carbohydrates, proteins, amino acid, vitamins and phenolic compounds.

Thin Layer Chromatographic study of the methanolic extract of *Lagenaria siceraria*, *Ocimum gratissimum* and *Moringa oleifera* were performed for the determination of saponin and phenolic compound using diosgenin and catechine as the reference standard respectively. The
results of TLC disclosed the existence of spaonin and phenolic mixes in the methanolic extracts of all three plant materials.

The methanolic and aqueous extract of *Lagenaria siceraria*, *Ocimum gratissimum* and *Moringa oleifera* were utilized for the pharmacological screening. In which the ED$_{50}$ was determined and it was 200 mg/kg bd. wt. for methanol (alcoholic) and aqueous concentrate of *L. siceraria*, 100 mg/kg bd. wt. for methanolic and aqueous extracts of *Ocimum gratissimum* and 500 mg/kg bd. wt. for methanolic and aqueous extracts of *Moringa oleifera*.

Methanolic and aqueous extract of all three plant materials was screened for the analgesic, anti-pyretic and inflammation curing potential on rats.

**Analgesic activity:**

Rats were taken for assessing analgesic activity of the sample. Albino wistar rats are alienated into eight clusters comprising six rats. Methanolic and aqueous extracts of *Lagenaria siceraria*(200 mg), *Ocimum gratissimum*(100) and *Moringa oleifera*(500 mg) were used for test. Gum acacia solution is used as control and pentazocine drug is used as standard.

At 180 min, *Lagenaria siceraria*aqueous extract displays a highly significant analgesic action (5.88 ± 0.012 seconds) while *Lagenaria siceraria*methanolic extract shows a moderately significant analgesic action (4.12 ± 0.012 seconds) when compared to standard drug (5.72 ± 0.071 seconds), while at 180 min aqueous extract of *Ocimum gratissimum* shows moderate analgesic activity (3.97 ± 0.006 seconds) while methanolic extract showed highly significant analgesic activity (5.81 ± 0.013 seconds). At 180 min both aqueous extract (5.91 ± 0.004 seconds) and methanolic extract (5.40 ± 0.014 seconds) of *Moringa oleifera* shows significant analgesic activity when compared to standard drug (5.72 ± 0.071 seconds), (p<0.05).

**Anti-pyretic activity:**

Rats of 150 to 250 g were taken for assessing analgesic activity of the sample. Albino wistar rats are separated into eight each sets containing six rats. Methanolic and aqueous extracts of *Lagenaria siceraria*(200 mg), *Ocimum gratissimum*(100 mg) and *Moringa oleifera*(500 mg)
were used for test. Brewer’s yeast suspension (15%) in normal saline is used as control and paracetamol drug is used as standard.

Methanolic extract (36.29 ± 0.23) and aqueous extract (38.26 ± 0.09) of *Lagenaria siceraria* does not show any noteworthy anti-pyretic action, when equated with the paracetamol (35.18 ± 0.21) at 180 minutes. Methanolic extract (36.23 ± 0.08) and aqueous extract (36.82 ± 0.51) of *Ocimum gratissimum* showed moderately significant fever reducing action, when equated with the paracetamol (35.18 ± 0.21) at 180 minutes. Methanolic extract (36.77 ± 0.31) and aqueous extract (38.29 ± 0.11) of *Moringa oleifera* do not show any noteworthy anti-pyretic action, when equated with the paracetamol (35.18 ± 0.21) at 180 minutes.

**Anti-inflammatory activity:**

Rats weighing 150 to 250 g were taken for assessing analgesic activity of the sample. Albino wistar rats are alienated into eight sets each containing six rats. Methanolic and aqueous extracts of *Lagenaria siceraria* (200 mg), *Ocimum gratissimum* (100 mg) and *Moringa oleifera* (500 mg) were used for test. Carrageenan: 0.1 ml of 1 % solution is used as control and drug diclofenac is used as standard.

The *Lagenaria siceraria* extracts condensed the carrageenan prompted rat paw edema by 37.50 % and 62.50 % correspondingly on oral dosing of 200 mg. The *Ocimum gratissimum* extracts of lessened the carrageenan prompted rat paw edema by 61.25 % and 36.25 % respectively on oral dosing of 100 mg, while the *Moringa oleifera* methanolic and aqueous extracts lessened the carrageenan prompted edema by 60.00 % and 33.75 % correspondingly on oral dosing of 500 mg, as equated to the untouched control set. Drug Diclofenac at 100 mg dose repressed the edema dimensions by 75.00 % (p<0.05).

The aqueous extracts of *Lagenaria siceraria*, *Moringa oleifera* and methanolic extract of *Ocimum gratissimum* showed good anti-inflammatory activity, hence they have been selected for the topical formulations. Four different formulations viz. TSF-1 (Hydrocarbon base), TSF-2 (water soluble base), TSF-3 (water miscible base) and TSF-4 (absorption base)
were formulated and evaluated for pH, spreadability, viscosity, skin irritation, release profile, drug release kinetics and stability.

The pH of preparations were gotten to be 9.003 ± 0.09 to 9.231 ± 0.032. No indications of edema and erythema were initiate after 48 h rats. Drug release showed that all formulation shows good release of medicament material from the formulated polyherbal formulation, except the formulation TSF-4 (13.97 %). While TSF-1 shows 65.42 %, TSF-2 shows 68.21 % and TSF-3 shows 95.55 % drug diffusion. From the outcomes, it is undoubtedly evident that productsexhibited desirable pH, spreadability and viscosity. The drug release kinetic study showed that theTSF-1, TSF-2 and TSF-3 followed zero order kinetics as best fit model. Whereas TSF-4 shows zero order kinetics based on the $r^2$ values.

By using solvent evaporation technique, the transdermal patches of aqueous extracts of *Lagenaria siceraria*, *Moringa oleifera* and methanolic extract of *Ocimum gratissimum* were developed using diverse concentrations of polymers. The developed patches batches (TP-1, TP-2, TP-2, and TP-4) were assessed for the weight uniformity, moisture content, tensile strength, thickness, folding endurance, moisture uptake, stability study, skin irritants, in-vitro drug diffusion and kinetics study.

From the outcomes, it is visibly apparent that the transdermal patches exhibited acceptable thickness (be 207 ± 0.19 µm to 280.1 ± 0.12 µm), weight uniformity (30.09 ± 0.32 to 30.19 ± 0.12 mg cm$^{-2}$), folding endurance (157.0 ± 0.30 to 210.0 ± 0.16), moisture content (3.12 ± 0.66 to 3.91 ± 0.61 %), moisture uptake (0.21 ± 0.19 % to 1.36 ± 0.20 %) and tensile strength (0.083 ± 0.12 kg cm$^{-2}$ to 1.3 ± 0.10 kg cm$^{-2}$). Edema and erythema were not developed after 48 h of application.

In-vitro drug release study of polyherbal transdermal patches were performed using Franz diffusion cell. The study showed 92.36 %, 98.26 %, 60.20 % and 94.26 % release after 480 minutes for TP-1, TP-2, TP-3 and TP-4 formulations respectively. From the results of the thickness, weight uniformity, folding endurance, tensile strength, moisture uptake, moisture
content, skin irritation and in-vitro drug diffusion TP-4 formulation was considered as optimized formulation and used in further studies.

All the conventional formulation and transdermal patch (TP-4) were exposed to stability studies as per guidelines of ICH. Results of studies of conventional formulations displays that there is no noteworthy deviations occur in the colour, odour and pH after storing at 25°C, 60 % RH, 40°C, 75 % RH for six months and 60°C, 80 % RH for four weeks. Thus it can be concluded that the formulations are stable.

Results of stability studies of optimized polyherbal transdermal patch TP-4 displays no significant changes occur after six month of storage at 40°C ± 2°C, 75 % ± 5 % RH. Thus it can be concluded that the formulation TP-4 is stable at 40°C, 75% RH.

All the conventional formulation and transdermal patch (TP-4) were screened for its anti-inflammatory potential. TSF-3 showed most-significant activity by reducing 66.67 % edema at 4th hour, while TSF-1, TSF-2 and TSF-4 showed 55.56 %, 59.26 % and 37.04 % reduction in paw edema, as equated to the untreated control set. The regular drug subdued the edema dimensions by 74.07 %.

The outcomes gottenspecify that the herbal transdermal patch had noteworthy anti-inflammatory potential. The herbal transdermal patch compact the edema carrageenan persuaded by 62.96 % as equated to the untreated group. Edema volume inhibited by Diclofenac sodium was 74.07 % (p<0.05).

Conventional formulation TSF-3 and transdermal patch formulation TP-4 showed nearly same reduction in edema at 4th hour i.e. 66.67 % and 62.96 % respectively.
Conclusion

The present work relates usually to a composition and method for managing pain and inflammation. More principally, the present development relates to the pain and inflammation managing composition, which contains extracts from plant species.

From the outcomes of phytochemical soundings, it can be settled that inorganic and calciferous matter present in the collected sample. Extractive values indicates that the plants contain a high amount of water soluble constituents as compared to alcohol soluble constituents. The plant contains moieties such as tannis, which shows various therapeutic effects as anthelmintics, antioxidants, antimicrobials and antivirals and for the cancer treatment. The plant parts also contains good % of phenolic contents. Polyphenols have numerous positive impacts on human wellbeing, like restraining the oxidization of proteins with low density, thereby decreasing the risk of heart disease. All phenolic mixes have calming and hostile to cancer-causing properties.

The powdered sample of fruit of *Lagenaria siceraria*, leaves of *Ocimum gratissimum* and *Moringa oleifera* showed better yield in aqueous extraction as compared to methanolic extraction. It concluded that the water soluble content are more in the plant extracts.

From the qualitative chemical investigation it can be concluded that the plants parts or extracts contains alkaloids, terpenoids, steroids, glycoside, tannins, flavonoids, carbohydrates, saponins, protein and amino acid.

From the outcomes of chromatographic study it can be settled that the aqueous and methanolic extracts of all three plants contains saponins and phenolic compounds.

The present research work concluded that three selected herbs; *Lagenaria siceraria*, *Ocimum gratissimum* and *Moringa oleifera* displayed significant analgesic and inflammation suppressing potential in their methanolic and aqueous extracts. However, significant (p<0.05) antipyretic activity was not shown by methanolic and aqueous extracts of *Lagenaria siceraria*, *Ocimum gratissimum* and *Moringa oleifera* showed significantly.
The aqueous extracts of *Lagenaria siceraria*, *Moringa oleifera* and methanolic extract of *Ocimum gratissimum* are selected for the development of formulations. All formulations were evaluated for their evaluation parameters i.e. determination of the pH, determination of spreadability, primary skin irritant studies, determination of viscosity, in-vitro diffusion study, release kinetic study and stability study and from the outcomes of all parameter it can be established that the all the formulation have fulfill the minimum quality control requirements for ointments.

The aqueous extracts of *Lagenaria siceraria* and *Moringa oleifera* and the methanolic extract of *Ocimum gratissimum* are used for the development of transdermal patches with different polymer concentrations. From the results of evaluation parameters i.e. weight uniformity, thickness, moisture uptake, folding endurance, moisture content, tensile strength, skin irritation, in-vitro drug diffusion, drug release kinetic study and stability study, it can be concluded that the prepared patches pass the quality control parameter and the from the stability study it can be concluded that the patches are stable at different storage conditions.

A polyherbal extract of *L. siceraria*, *Moringa oleifera* and *Ocimum gratissimum* herbs in conventional topical semisolid preparations having water miscible base showed significant anti-inflammatory action as equated to absorption and oleaginous ointment bases.
**Recommendation**

- Plants utilized as prospective medicinal basis of medicine for period immemorial. It estimated that 20,000 plants species were used for medicinal purposes.

- Roughly from 119 plant species, 74% inferred medications was found as a consequence of compound studies to segregate the dynamic chemical moiety which was responsible for their medicinal use.

- People gained valuable experiences by collecting and using medicinal plants and handed down their knowledge to their generations.

- Ayurveda has well-known treaties known as Charak Samhita and Susrut Samhita, the oldest and very first written document of Ayurveda (900 BC)

- The traditional medicine used in India known popularly as the Indian arrangement of prescription incorporates Siddha, Ayurveda, Unani and Naturopathy.

- Herbal therapy gives logic means for curing of many internal ailments which are considered to be obstinate and incurable in other medicinal systems.

- The R&D department of various herbal industries investing on research and development of OTC remedies. The isolation and characterization of herbal extracts became a major part of pharmacopoeias.

- Herbal remedies popularized because of their efficacy, easy obtainability, low price and somewhat being lacking of serious lethal effects.

- Herbal technology is the biggest revenue earner in India after information technology. Plant-based drugs or drug products are continuously progressively favored in medicinal science.
WHO has stressed on the need to guarantee the quality control of herbs and home grown plans by utilizing current techniques.

**Future strand**

- Findings of these studies conclusively demonstrate that the topical novel drug delivery technology for herbal extracts can knock the market because of the reasons of many pharmaceutical problems occurred in conventional delivery systems. That either may not appear or less troublesome for the manufacturers and also decipher many issues of patient's acceptance and compliance.

- The present invention provides a conventional and novel composition which transdermally administered plant extracts for ameliorating pain and inflammation.

- This exploration report has investigated a lot of people new things on the relationship between pharmaceutical natural arrangements.

- Then again, from the essential examination discoveries, the scientist has distinguished that further isolation of active moiety and its approval will help the further specialist for pharmaceutical measurements structure advancements, for example, tablets which are steadier than semisolid preparations.

- In comparable ways, research has distinguished and investigated diverse issues and concerns from the survey of written works and essential exploration discoveries that have huge qualities for future and further study both from scholastic and industrial purpose of perspectives.
**Limitation**

- The poor anti-inflammatory activity in absorption and oleaginous ointment bases may be due to absorption of the aqueous polyherbal extract thereby not allowing the herbal constituents to release from the dosage forms in response to the strong bonding of aqueous polyherbal extract and the hydrophilic groups of lanolin present in the absorption ointment base and high hydrophilicity of polyethylene glycols in water soluble ointment base.

- Such problems of selection of different semisolid ointment bases can be worked out by developing a topical novel system of drug delivery like transdermal patch of polyherbal extract.

- In the light of the similar pharmacological activity in both conventional and novel delivery systems, it is therefore accomplished that the novel drug delivery system of herbal medicines may deal with the limitations of the traditional drug delivery systems.