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FORMULATION AND EVALUATION OF PHYTOSOAMAL GEL OF (AZADIRACHTA INDICA) NEEM

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ABSTRACT

Phytosomes of neem leave extract has been successfully formulated in this procedure firstly *Azadirachta indica* leaves were morphological Characterized. Neem leave powder was subjected for various physiochemical analysis then extracted out methanolic fraction of neem leaves, yield of the extract which was 6.34 %. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present. then extracted out methanolic fraction of neem leaves, yield of the extract which was 6.34 %. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present. Total phenolic compound (TPC) was 0.756mg/100mg of gallic acid equivalent of dry extract sample, Total alkaloid content was atropine equivalent 0.632mg/100mg. Five different type of phytosome formulations were formed using different ratio of Phosphatidylcholine and Cholesterol (4:1), (2:1), (4:3), (1:1) and (1:1.25). These formulations were characterized under various parameters like yield, drug content, particle size and Encapsulation Efficiency. For all formulation yield was 88.32 % to 98.91%, drug content was 90.21% to 97.52 %, Mean Particle Size(nm) about 700 nm and Encapsulation Efficiency was 78.67% to 95.34%. Drug: Excipient Compatibility confirmed by FT-IR studies. All five phytosome formulations were incorporated with gel and evaluated under the various parameter, pH of all formulations was observed between 6.8 to 7.3 and Spreadability between 5.6 to 7.9 cm. % drug content between 98.9 % to 101%, and viscosity between 98 to 115 centi poice (cp) and % permeation between 83.2 % to 92.7 %. But on the basis of drug release kinetics F-3 formulation was very good because its % drug release was 97.913% followed Higuchi Kinetic Model. With the 95.56% drug release F-2 formulation was good both follow First order Kinetics.

Keyword: *Azadirachta Indica,* Physiochemical, Phytosome, Gel, Neem.

I. INTRODUCTION

Azadirachta indica is popularly known as Indian neem or margosa tree. It's been extensively used in ayurveda, unani and homoeopathic medicine since time immemorial. In Sanskrit a "good health" condition is expressed as "Nimba", which on due time derived in to "Neem", further the tree is considered as "Sarvaroga nivarini" means cure all ailments. In Ayurveda neem is known as "Arishtha" meaning 'reliever of sickness'. The tree is still regarded as "village pharmacy" or "Divine tree" due to presence of medicinal properties in India. If the developing countries are considered more than 80% of the population is believed to be dependent on medicinal plants for curing various diseases or disorders. Further, the total trade in medicinal plants in India during 2004-05 has been 4,530 crore. India ranks second in the world in terms of the volume and value of medicinal plants export.

Fig 1: Neem is one of the indigenous medicinal plants

India which possess medicinal properties in each and every part viz., roots, seeds, flowers, bark, leaves, fruit pulp etc.. Neem is one of the examples of complementary medicine through phytotherapy. Each of the plant part has been used in the Indian Ayurvedic and Unani systems of medicine and has become a cynosure of modern medicine.

In Ayurvedic literature neem is well known for its medicinal properties viz., Neem bark is cool, bitter, astringent and acrid. In addition to this, it is used to cure tiredness, cough, fever, loss of appetite, worm infestation etc. It also

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heals wounds and vitiated conditions of kapha, vomiting, skin diseases, excessive thirst and diabetes. Along the bark, chemical compounds present in the leaves are reported to be valuable for eye disorders and insect poisons. It treats Vatik disorder and anthelminthic'. In the view of its immense utilities, this review summarizes the wide range of medicinal uses, pharmacological activities, biological activities of neem tree and its compounds and their chemistry.

Human society used the medicinal plantsto combat diseases, since the dawn of civilization. A number of alternative medicine systems exist in the eastern region of the Mediterranean. For thousands of years nature has been a source of medicinal agents and based on.

their uses in traditional medicine an impressive number of modern drugs have been isolated from natural sources. In fact, plants are rich source of different types of medicines because they produce a diverse range of bioactive molecules. In the pharmaceutical industries natural products play an important role in drug development programs, therefore, over 50%of all modern clinical drugs are of natural product origin. In the addition of the importance of synthetic medicinal chemistry, there is huge interest in herbal medicine, there has been a revival of interest in herbal medicines to control major diseases and to discover new molecular structures as lead compounds from the plant kingdom.

Fig 2:

Azadirachta indica A. Juss. is a tree of small to medium-sized around 18 m, tall up to 15-30 m, with large crown up to 10-20 m diameter. The leaves are light green simply pinnate alternate with 20-40 cm long. The flowers are pentamerous, small, white or pale yellow, slightly sweet and bisexual. The plants fruits are greenish yellow to yellow or purple and have one or two seeded drupe, ellipsoidal, 1-2 cm long. They are greenish when ripe and their seeds are ovoid or spherical. Neem's leaves, seeds, bark, roots, fruits and oil have become a cynosure.

II. MATERIALS AND METHODS

Preliminary Work

Collection of Plant material: The leaves of *Azadirachta indica* was collected in the month of January from Bhopal, region Madhya Pradesh, India.

Drying and Size Reduction of Plant Material: leaves of *Azadirachta indica* were dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of leaves was passed through sieveNo. 18 to maintain uniformity and stored in cool and dry place for study.

Screening of Powder (Physiochemical Analysis): Physiochemical screening of powdered leaves under the parameters Loss on Drying, Total Ash Value, Acid Insoluble Ash Value, Water Soluble Ash Value and Foaming Index was done by the standard reported methods.

Preparation of *Azadirachta indica* leaves extract

(a) Extraction of leaves of *Azadirachta indica:* Extraction of leaves of *Azadirachta indica* was done by Soxhlet extraction method.

Soxhlet Extraction: Soxhlet apparatus was used for the solvent extraction and methanol was selected as a solvent for extraction while petroleum ether was used for defatting of waxy materials.

Phytochemical Screening:

Extract obtained after extraction were subjected for phytochemical screeningto determine the presence of following various phytochemical present in the extracts.

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Quantitative studies of phytoconstituents

Estimation of total phenol content:

The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 2 ml of extract and each standard was mixed with 1 ml of Folin- Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l)of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer

Estimation of total alkaloids content:

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1-, 2-, 3- and 4-ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in thesame manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg ofextract.

Preparation of Phytsomes of *Azadirachta indica* extract

Accurately weighed quantity of phosphatidylcholine and cholesterol were dissolved in 10 ml of chloroform in round bottom flask (RBF) and sonicated for 10 min using bath sonicator. Organic solvent removal is done by Rotary evaporator (45-50°C). After complete removal of solvent thin layer of phospholipids mixture was formed. This film was hydrated with methanolic extract of neem leaves in rotary evaporator (37-40°C for 1 hour). After hydration, mixture of lipid and plant extract was sonicated for 20 minutes in presence of ice bath for heat dissipation. Then prepared phytosomes were filled in amber colored bottle and stored in freezer (2-8^{0}C) until used.

Characterization of Phytsomes of *Azadirachta indica* extract

A. Visualisation:

The morphology of phytosomes was observed by digital microscopy, transmission electron microscope and scanning electron microscope.

Digital Microscopy: Phytosome formulation shaken in distilled water and viewed under digital microscope at 400X objective lens. All the batches prepared were analyzed for particle size by optical microscope.

SEM Analysis: Approximately 5 µL of the phytosomal suspension was transformed to a cover slip, which in turn was mounted on a specimen tab. The samples were allowed to dry at room temperature. Then the particle size of the formulation was viewed and photographed using Scanning Electron Microscope (Sigma, Carl Zeiss).

B. FTIR:

spectral data were taken to ascertain the structure and chemical stability of extract, PC and phytosome.Spectral scanning was done in the range between 4000 and 500 cm^{-1}

C. In-Vitro Drug Release Studies:

The in vitro dissolution studies were carried using USP - 34 paddle type dissolution apparatus. 50 mg neem extract loaded phytosomes were placed in a dialysis bag and introduced into100 ml dissolution medium of buffer solution pH 7.4 maintained at 37± 0.5 ºC at a rotation speed of 50 RPM.1 ml of aliquots was withdrawn at predetermined time intervals and an equivalent volume of fresh medium was replaced to maintain sink condition. The aliquots were diluted and analyzed spectrophotometrically at 330nm to determine the concentration of drug present. The readings were taken thrice and the average reading was taken for further calculation.

Accelerated stability studies

The above prepared samples were kept in sealed vials for 7 days at 40 ^{o}C and 75% RH.

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Preparation of Neem extract loaded phytosomal gel

Dissolve different concentrations of HPMC in ethanol and propylene glycol in water were mixed together usinga magnetic stirrer, at 25 rpm. By keeping Neem extract loaded phytosome concentration constant in all the formulations. The Neem extract loaded phytosome was poured into the polymer solution. The solution was kept under stirring and then the pH was adjusted using 0.1M NaOH and the formulated gel was taken for further analysis.

Evaluation of prepared Gel

Physical examination: The formulation was manually examined to check any variations in the color, odor, and texture.

Determination of pH: pH of each formulation was determined by using pH meter (pH meter Toshconcl 54+) which was calibrated before with buffer solutions of pH 4, 7 and 9.

Determination of Viscosity: Viscosity of each formulation was determined using Brookfield viscometer with spindle at room temperature and at 5, 10, 20, 50 and 100 rpm.

Drug content: 0.2 gm of the gel formulation (equivalent to 10 mg of drug) was taken in 100 ml volumetric flask which contains 20 ml of phosphate buffer pH 7.4 and sonicated for 15 minutes. Volume was made upto 100 ml. 1ml of above solution was further dilute to 10 ml by using phosphate buffer of pH 7.4. The resultant solution was subjected to UV spectrophotometric analysis at 330 nm and the absorbance was noted down.

Spreadability: To determine spreadability of the gel formulations, two glass slides of known standard dimensions are selected. Formulation whose spreadability to be determined was place on one slide and then otherslide was kept over its top such that the gel is sandwiched between the two slides. The slides were pressed uponeach other so as to displace any air present, and the adhering gel was wiped off. The two slides were placed ontoa stand such that only the lower slide is held firm by the one opposite fangs of the clamp clips and allows the upper slide to slip freely over it by the force of weight tied Tie the 20-gm weight to the upper slide carefully. Thetime taken by the upper slide to completely detach from the lower slide was noted. The spreadability was calculated by using the following formula.

$$s = m \frac{l}{t}$$

Value 's' is spreadibility, m is the weight tied to the upper slides, l is the length of glass slide, and t is the time taken.

Active Compounds of Neem

Azadirachta indica shows therapeutics role in health management due to rich source of various types of ingredients. Different parts of the Neem tree contain numerous types of ingredients such as azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimibidol, sodium nimbinate, gedunin, salannin and quercetin. Leaves of Neem tree contain ingredients such as nimbin, nimbanene, nimbandiol, ascorbic acid, 6-desaacetylnimbinene, nimbolide, n-hexacosanol and amino acid, 7-desaacetyl-7- benzoylgedunin, 7-desaacetyl-7-benzoylazadiradione, 17hydroxyazadiradione, and nimbiol [5, 6, 7]. Quercetin and Beta-sitosterol are polyphenolic flavonoids were purified from Neem fresh leaves and they are known to have antibacterial and antifungal properties [1]. Seeds of Neem tree hold valuable constituents incuding gedunin and azadirachtin. The active constituent of Azadirachta indica show therapeutic implications in the modulation of cell signaling pathway involved in the management of cancer. Neem and its active ingredient play a role in prevention and treatment of tumor due to its broader pharmacological activities. Neem tree extracts have been extensively used in health management since ancient times and have a variety of health promoting properties.

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7-benzoylazadiradione, 17hydroxyazadiradione, and nimbiol [5, 6, 7]. Quercetin and Beta-sitosterol are polyphenolic flavonoids were purified from Neem fresh leaves and they are known to have antibacterial and antifungal properties [1]. Seeds of Neem tree hold valuable constituents incuding gedunin and azadirachtin. The active constituent of Azadirachta indica show therapeutic implications in the modulation of cell signaling pathway involved in the management of cancer. Neem and its active ingredient play a role in prevention and treatment of tumor due to its broader pharmacological activities. Neem tree extracts have been extensively used in health management since ancient times and have a variety of health promoting properties.

CHEMISTRY OF NEEM COMPOUNDS

Natural compounds present in neem are triterpenes or limonoids. New limonoids are still being discovered in neem. Azadirachtin, salannin, meliantriol and nimbin

are well known. The bitter constituent, the nimbin contains an acetoxy, a lactone, an ester, a methoxy and an aldehyde group. Nimbidin contains sulphur.

Bark

The bark exudes a clean bright amber coloured gum which is collected in small tears or fragments. It contains a bitter alkaloid named “margosine”. Leaves also bitter principles but in small quantity which are much more soluble in water. This substance is a hydrate of the resin.

Flowers

Flowers have been found to contain a flavonoid. Nimbicetin is identical to kaempferol. In the dried bark the same bitter components as in the seed oil have been found and in the pericarp of the fruit a bitter principle bakayanin was found.

Neem oil

Neem oil contains Sulphur 0.427%; a very bitter yellowish substance obtained from the alcoholic extract of the oil, whichis supposed to be an alkaloid; resins; glucosides and fatty acids

Seeds

Meliacins found in the seeds include gedunin , 7-desacetylgedunin, desacetylnimbin and azedarachtin. The seed oil mainly contains nimbidin, nimbin and nimbinin, which also occur in the stembark.

Toddy

The toddy or sap contains glucose, sucrose, gums and coloring matter.

III. MEDICINAL USES

Ayurveda

Neem tree has occupied a prominent place in the traditional Ayurvedic medicine in India from time immemorial. Neem bark, leaf extracts and neem oil have been under use as folk medicine to control various problems viz., leprosy, intestinal helminthiasis, constipation, etc. Further, it plays vital role in treating rheumatism, chronic syphilitic sores and indolent ulcers. Neem oil is well known to control various skin problems. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis. The root bark and young fruits are used as an alterative, antiperiodic and as a tonic. Green twigs are used as toothbrushes for cleaning teeth and as a prophylactic for mouth and teeth complaints. The bark, gum, leaf and seed are used in snake bite and scorpion sting. The bark is used as a bitter tonic, astringent, antiperiodic, antipyretic and against nausea and vomiting. Gum is demulcent tonic in catarrhal affections. Leaves are used as poultice for boils. Decoction of leaves used as an antiseptic in ulcers and eczema. Dry flowers are stomachic. Seed oil is a stimulant, antiseptic, alterative in rheumatism and skin diseases. Berries are purgative, emollient and anthelminthic. An extract of leaves is used in toothpastes. Neem oil is effective in the treatment of leprosy and skin diseases.

Homoeopathy

Used against rheumatic pains. Pain in sternum and ribs, in the extremities and aches in hands and toes. Also used against eczema, pemphigus and scabies.

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Unani

Neem finds use as a resolvent and blood purifier. Leaves expel wind, heal ulcers in urinary passages. Used as an emmenagogue and in skin diseases.

Fruit is used as an astringent and in leprosy and bronchitis.

Immunostimulant activity

Various studies have revealed that the aqueous extract of leaf and bark possesses anticomplement and immunostimulant activity. Neem oil has been shown to possess activity by selectively activating the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge.

Hypoglycaemic activity

Neem leaf extracts showed promising results in decreasing blood sugar level and prevents adrenaline as well as glucoseinduced hyperglycaemia. Recently, hypo glycaemic effect was observed with leaf extract and seed oil in normal as well as alloxan-induced diabetic rabbits.

Antiulcer effect

Neem leaf and bark aqueous extracts produce highly potent antiacid secretory and antiulcer activity. A significant antiulcer effect was observed with nimbidin in preventing acetylsalicylic acid, indomethacin, stress or serotonin-induced gastric lesions as well as histamine or cysteamine-induced duodenal ulcers.

Antifertility effect

Neem seed and leaf extract possess the chemical constituents which can act as anti-fertility sources. Studies on this concept have revealed that intravaginal application of neem oil, can prevent pregnancy, thereby stating it as a novel method of contraception. NIM- 76, a refined product from neem oil, was studied in 10 human volunteers, where intravaginal application before sexual intercourse could prevent pregnancy with no adverse effect on vagina, cervix and uterus, further, the study revealed that intrauterine treatment is safe. Aqueous extracts of seeds and leaves contain sodium nimbinate (triterpene) which showed antifertility activity.

Antimalarial activity

Neem seed and leaf extracts are effective against both chloroquin-resistant and sensitive strain malarial parasites. One of the neem's components, "gedunin" (a limonoid), is as effective as quinine against malaria. Malaria is one of the pandemic diseases causing millions of deaths every year in India and several other countries. China has adopted neem in a big way to reap the antimalarial effects of neem. The antimalarial formulation "Quinahausa" prepared in China will be available in India as well. Neem oil treated mosquito nets and mosquito-repellent cheap tablets are also becoming popular, due of growing problems of resistance to conventional treatments, it is becoming more and more difficult to control malaria. Clinical trials have been conducted to check the efficacy of neem extracts to control hyperlipidemia in a group of malarial patients severely infected with P. falciparum. The lipid level, especially cholesterol, was found to be lower during therapy when compared to non-malaria patients.

Antifungal activity

From time immemorial it is believed that Neem is effective against certain fungi that infect the human body. Some important fungi against which neem preparations have been found to be effective are: athlete's foot fungus that infects hair, skin and nails; a ringworm that invades both skin and nails of the feet, fungus develops in intestinal tract, bronchi, lungs, and mucous membranes and a fungus that is part of the normal mucous flora that can get out of control leading to lesions in mouth (thrush), vagina, etc. Extracts of neem leaf, neem oil seed kernels are effective against certain fungi including Trichophyton, Epidermophyton, Microspor, Trichosporon, Geotricum and Candida.

Antibacterial activity

Neem derives compounds especially Azadirachtin is well known for its role as antibacterial agent. It is a complex tetranorteiterpenoid limonoid present in the seeds as well as leaves which is highly responsible for toxic effect on microbes. Extracts of the leaves, seed and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including M. tuberculosis and streptomycin resistant strains. In vitro, it inhibits Vibrio cholerae Klebsiella pneumoniae, M. tuberculosis and M. pyogenes.

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Antimicrobial effects of neem extract have been demonstrated against Streptococcus mutans and S. faecalis. Apart from azadirachtin, other components such as nimbidin, nimbin, nimbolide, gedunin, mahmoodin, margolone, and cyclic trisulfide contribute to the anti-bacterial activity of neem. Further, neem extracts are a ray of hope to cure deadly diseases viz., Chagas disease in Latin America which was uncontrolled by any other means of medicines. This disease is caused by a parasite which is carried by an insect called kissing bug. Research has shown that feeding neem to the bugs not only frees them of parasites, but azadirachtin prevents the young insects from molting and the adults from reproducing.

Antiviral activity

Aqueous leaf extract offers antiviral activity against Vaccinia virus, Chikungunya and measles virus. Nimbin and nimbidin have been found to have antiviral activity. They affect potato virus X, vaccinia virus, and fowl pox virus.

Anticancer activity

Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7, 12-dimethylbenz[a] anthracene (DMBA), as revealed by reduced incidence of neoplasm. Conducted a study in chemoprotective neem compounds viz., azadirachtin, nimbolide and limonoid enrich extracts on models of buccal carcinogenesis in hamsters. Overall studies were tested positive to reduce the expression and cell proliferation antigens. Further, researchers have shown prominent anti-cancerous activities from limonoidderived compounds from neem. Amongst these, both 1Odeacetylohchinolide B and 15-O-deacetylnimbolindin-B are proved to be beneficial to hinder cell growth in human cervical adenocarcinoma. A very recent study discovered that alkaloid-derived limonoid, azadiramide-A, is primarily found in Neem leaf ethanolic extracts, showed to stop cell growth and induce apoptosis in both the estrogen independent MDAMB-231 and estrogen dependent MCF-7 cell lines of breast cancer in human beings.

Antioxidant activity

The antioxidant activity of neem seed extract has been demonstrated in vivo during horse- grain germination which is associated with low levels of lipooxygenase activity and lipid peroxides. An antioxidant principle has also been isolated, which is a potent inhibitor of plant lipooxygenases. Antioxidants derived from neem is simple and cost effective way to supplement with natural

Anti-diabetic effect

Diabetes is one of the major chronic degenerative disorders now the world is facing. According to the health survey conservatively by 2030 there is expectancy for diabetes to be the 11th leading cause of death. Keeping in view of the severity of disease searching the ways for lower cost treatments must be need of hour. Among the various methods and pharmaco therapies being developed, the use of Neem extracts has steadily grown in interest. Several studies carried out in induced-diabetic rat models have revealed rescue of the G6PD when treated with Neem extracts.

Effect on central nervous system

Varying degrees of central nervous system (CNS) depressant activity in mice was observed with the leaf extract. Fractions of acetone extract of leaf showed significant CNS depressant activity. Leaf extract up to a dose of 200 mg/kg body weight produces significant anxiolytic activity in rats. The crude ethanolic extracts of stem bark and root bark showed hypotensive, spasmolytic and diuretic activities.

Other activity

The gum from bark is a stimulant and demulcent tonic. It possesses antileprosy, antispirochaetal, and immenagogue properites. Neem is widely used for treating fevers. It has anti-pyretic (fever-reducing) property. Apart from these benefits, neem products also have analgesic (pain-relieving) and antiinflammatroy effects, i.e. for most common ailments neem can provide organic, cheap, easily available and local medicines, thereby neem can bring sustainable livelihood to many people especially in rural and tribal regions.

IV. RESULT & DISCUSSION

Morphological Characterization of *Azadirachta indica* leaves: Leaves of *Azadirachta indica* were green in color, bitter in taste, Length – 1.5-3cm, Width -1-1.5cm in size, ovate in shape and Rough outer periphery.

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Physiochemical analysis of *Azadirachta indica* leaves powder

Table 1: Physiochemical analysis of powder of *Azadirachta indica* leaves

S. No.	Parameters	Observation (%)
1	Total ash value	9
2	Loss on drying	1.2
3	Acid insoluble ash value	2.9
4	Water soluble ash value	1.6
5	Foaming index	6 (ml)

Extract of Azadirachta indica: Extractive values of Pet. ether extracts of Azadirachta indica were % Yield (2.91% w/w) , Dark green Color, greasy in Consistency and methanol extracts of Azadirachta indica were % Yield (6.34% w/w), Dark green Color, semi solid Consistency.

Table 2:

S. No.	Chemical Tests	Ethanolic extract
1	**Carbohydrates** i) Molisch's Test ii) Fehling's Test iii) Benedict's test	 (+) (-) (+)
2	**Tannins** i) with 5%ferric chloride solution ii)with 10% aqueous Potassium dichromate solution iii) with 10% lead acetate solution	 (-) (-) (-)
3	**Alkaloids** i) Dragendorff's Test ii) Mayer's Test iii) Hager's Test	 (-) (+) (+)
4	**Glycosides** i) Borntrager's Test ii) Legal Test iii) Baljet Test	 (+) (–) (-)
5	**Flavonoids** i) Shinoda's Test ii) Alkaline reagent test iii) Lead test	 (+) (+) (+)
6	**Steroids and Sterols** **i) Libermann-Burchard Test** **ii) Salkowski Test**	 **(-)** **(-)**
7	**Proteins and Amino Acids** **i) Biuret Test** **ii) Ninhydrin Test** **iv) Millon's Test**	 **(–)** **(-)** **(+)**

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(+) = Present, (–) = Absence

Estimation of total phenol and alkaloid content in extract of *Azadirachta indica* leaves

Total Phenolic content estimation (TPC): Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.019x + 0.020, R^2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 3: Preparation of Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	10	0.226
3	20	0.412
4	30	0.614
5	40	0.803
6	50	0.966

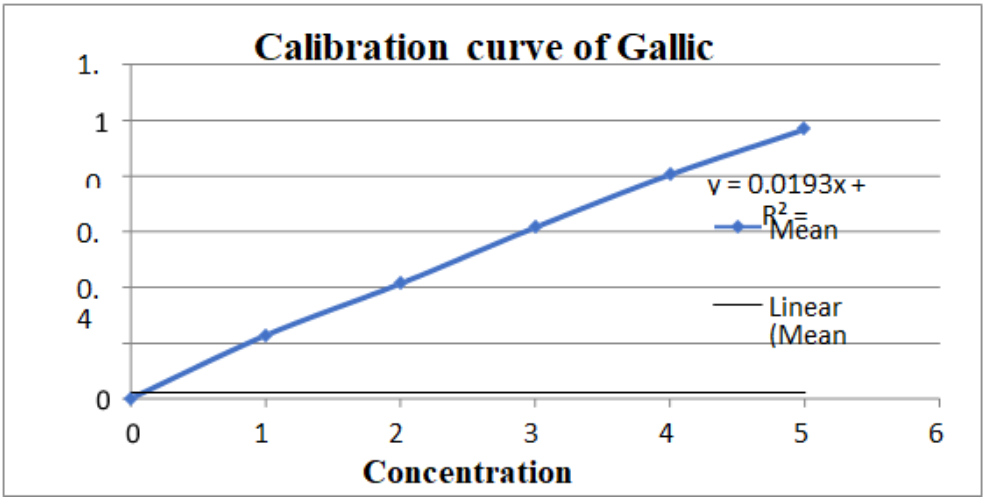

Fig 3:

Total alkaloid content estimation (TAC): Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: y = 0.008x + 0.010, R^2= 0.999, where X is the Atropine equivalent (AE) and Y is the absorbance.

Table 4: Preparation of calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean Absorbance
1	**0**	**0**
2	40	0.352
3	60	0.514
4	80	0.679
5	100	0.845
6	120	0.997

Table 5: Estimation of total phenolic and alkaloid content *Azadirachta indica* extract

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Methanol	0.756	0.632

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Formulation of Phytosome of neem leaves extract:

Five different type formulations formed using different concentration of drug and polymer.

Table 6: Formulations formed using different concentration of drug and polymer

Formulation code	Neem leaves extract (mg)	Phosphatidylcholine (PC) (mg)	Cholesterol (CL)(mg)	Phytosome PC:CL
F-1	200	100	25	4:1
F-2	200	100	50	2:1
F-3	200	100	75	4:3
F-4	200	100	100	1:1
F-5	200	100	125	1:1.25

Characterization of Neem Extract Loaded Phytosome

Table 7: Characterization of Neem Extract Phytosome

Batch Code	Yield (%)	Drug Content (%)	Mean Particle Size(nm)	Encapsulation Efficiency (%)
F1	89.12 ± 0.05	90.21 ± 0.21	732 ± 13	78.67 ± 1.52
F2	88.32 ± 0.08	92.35 ± 0.76	694 ± 29	84.27 ± 0.81
F3	98.91 ± 0.03	97.52 ± 1.90	637 ± 17	95.34 ± 0.64
F4	94.45 ± 1.06	91.41 ± 1.63	713 ± 44	92.52 ± 1.30
F5	93.48 ± 1.95	90.44 ± 1.02	694 ± 21	92.19 ± 1.68

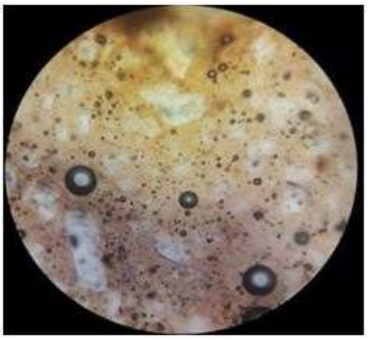

Figure 4: Microscopic observation of optimized batch F-3

Drug: Excipient Compatibility by FT-IR Study

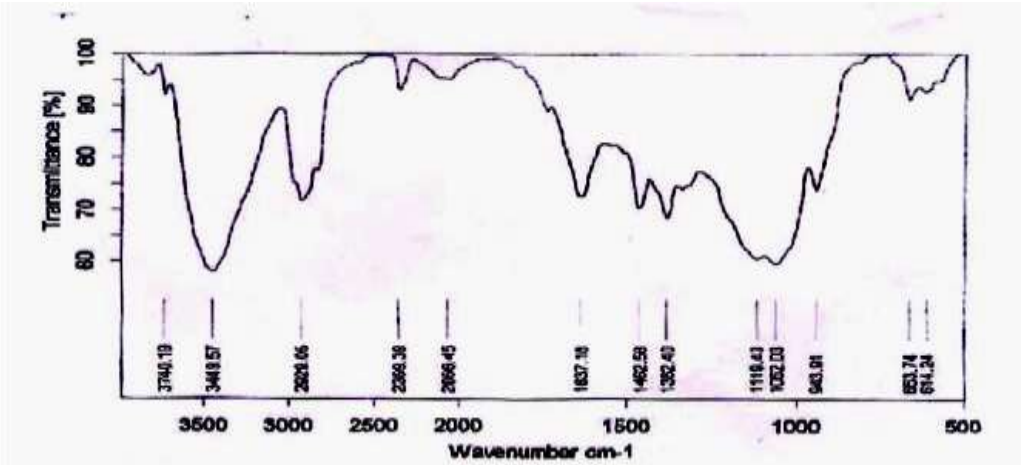

Figure 5: FT-IR of Neem Leaves Extract

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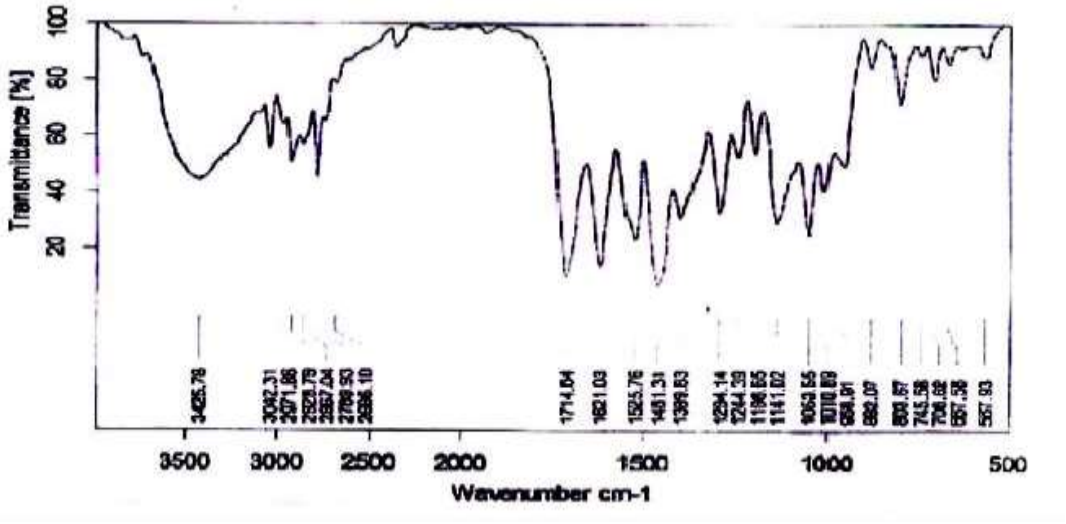

Figure 6: FT-IR of Neem Leaves Extract and Additives

Table 8: FT-IR Peaks of Neem Leaves Extract

Standarded Peaks (Cm^{-1})	Observed Peaks (Cm^{-1})	Peak Assignments
3050-3500	3449	O-H str
3000-2840	2829	CH3str
1650-1600	1637	C=C str
1750-1700	1720	C=O str (Carboxylic acid)
1392-1366	1362	N-H str

Str. = Stretching

Formulation of topical gel of Neem extract loaded Phytosome

Table 9: Formulation of Topical Gel of Neem Extract Loaded Phytosome

Formulation	F-1	F-2	F-3	F-4	F-5
Phytosome(g)	0.4	0.4	0.4	0.4	0.4
HPMC(g)	0.5	0.5	0.5	0.5	0.5
Ethanol (ml)	5	5	5	5	5
Propylene glycol (g)	1	1	1	1	1
Distilled water (g)	3.1	3.1	3.1	3.1	3.1

Evaluation of Neem Extract Loaded Phytosome gel

Table 10: Physical Evaluation of Neem Extract Loaded Phytosome gel

Formulation code	Clarity	Odor	Phase Separation	Wash ability	Homogeneity	Grittiness
F-1	Clear	No	No	Washable	Yes	No
F-2	Clear	No	No	Washable	Yes	No
F-3	Clear	No	No	Washable	Yes	No
F-4	Clear	No	No	Washable	Yes	No
F-5	Clear	No	No	Washable	Yes	No

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Table 11: Evaluation of Neem Extract Loaded Phytosome gel

Formulation code	pH	Spread-ability(cm)	% Drug Content	Viscosity(cp)	% Permeation
F-1	6.9	5.6 ± 0.3	99.9 ± 1.2	110 ± 1.8	83.2%
F-2	6.8	6.8 ± 0.2	99.6 ± 2.1	113 ± 2.0	86.3%
F-3	7.1	7.1 ± 0.6	98.9 ± 6.1	115 ± 1.2	92.7%
F-4	7.3	7.6 ± 0.2	99.8 ± 5.7	100 ± 0.8	91.0%
F-5	7.1	7.9 ± 0.6	101 ± 0.2	98 ± 2.6	90.1%

In-Vitro Drug Release Profile of Neem Extract Loaded Phytosome gel

Table 12:

Time (T) (Hr.)	%C. R. F-1	%C. R. F-2	%C. R. F-3	%C. R. F-4	%C. R. F-5
0	0	0	0	0	0
1	17.249	19.62	21.6	22.68	24.3
2	29.835	31.68	33.12	30.726	33.255
4	32.34	39.68	44.64	41.876	37.399
6	44.566	48.7	49.692	48.227	42.645
8	50.931	59.22	60.405	49.932	50.979
10	60.57	65.62	70.276	55.785	55.758
12	78.541	82.18	73.72	61.489	60.922
16	81.49	84.6	81.681	67.403	64.853
18	84.273	88.56	87.011	72.808	69.164
20	88.329	93.18	93.092	76.621	74.577
24	92.765	95.56	97.913	81.533	80.018

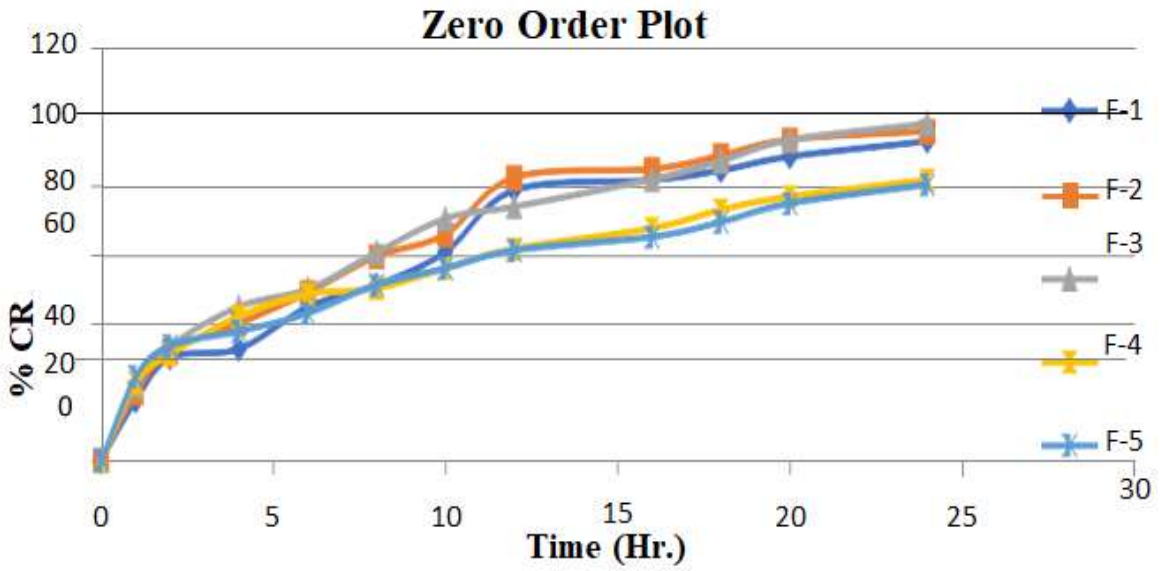

Figure 7: Zero order plots of all five Formulations

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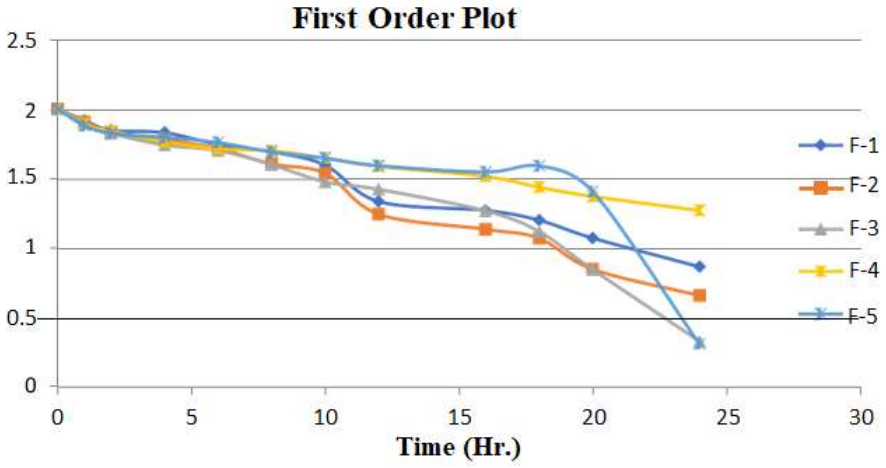

Figure: 8: First order plots of all five Formulations

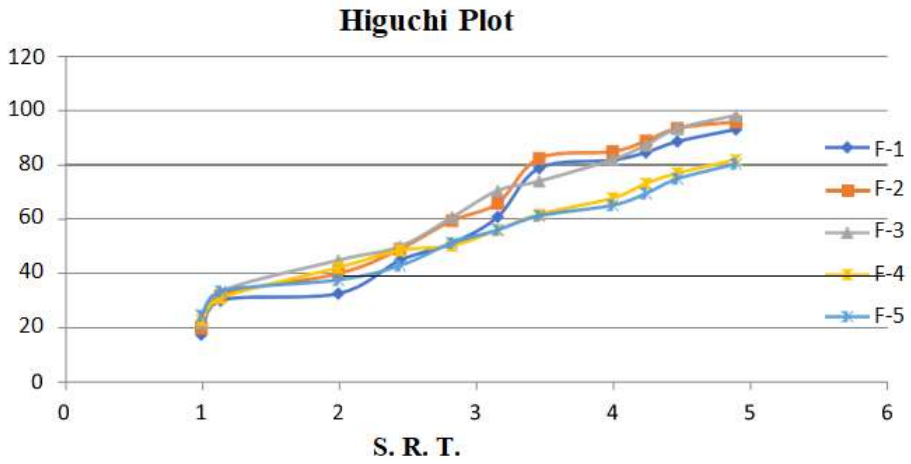

Figure 9: Higuchi plots of all five Formulations

Figure: R^2 Values of different of formulations in different type of plots

Table 13:

Formulation code	Zero Order	First Order	Higuchi
F-1	0.812	0.982	0.973
F-2	0.893	0.984	0.981
F-3	0.898	0.935	0.990
F-4	0.871	0.977	0.981
F-5	0.872	0.650	0.974

Stability study of Neem extract loaded Phytosome gel

Table 14: Stability of Neem extract loaded Phytosome gel

Formulation code	Phase separation		pH		Drug content (%)	
	4^oC	40^oC	4^oC	40^oC	4^oC	40^oC
F-1	No	No	7.1	7.3	100 ± 1.6	98 ± 1.6
F-2	No	No	7.2	7.1	100 ± 1.1	99 ± 1.3
F-3	No	No	7.0	7.1	99 ± 1.3	99 ± 1.1

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F-4	No	No	6.5	7.1	98 ± 0.9	96 ± 0.6
F-5	Yes	Yes	6.9	6.2	99 ± 0.3	94 ± 1.1

Discussion

Phytosomes of neem leave extract has been successfully formulated in this procedure firstly *Azadirachta indica* leaves were morphological Characterized. Neem leave powder was subjected for various physiochemical analysis like total ash value (9), Loss on Drying (1.2), Acid Insoluble Ash Value (2.7), Water Soluble Ash Value (1.6) and Foaming Index (6 ml) and then extracted out methanolic fraction of neem leaves, yield of the extract which was 6.34 %. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present.

Estimation of total phenol and alkaloid content in extract of *Azadirachta indica* leaves. Total phenolic compounds (TPC) was 0.756mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.019x + 0.020$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total alkaloid content was atropine equivalent 0.632mg/100mg using the equation based on the calibration curve: $y = 0.008x + 0.010$, $R^2 = 0.999$, where X is the Atropine equivalent (AE) and Y is the absorbance.

Five different types of phytosome formulations were formed using different ratio of Phosphatidylcholine and Cholesterol (4:1), (2:1), (4:3), (1:1) and (1:1.25). These formulations were characterized under various parameters like yield, drug content, particle size and Encapsulation Efficiency. For all formulation yield was 88.32 % to 98.91%, drug content was 90.21% to 97.52 %, Mean Particle Size(nm) about 700 nm and Encapsulation Efficiency was 78.67% to 95.34%. Drug: Excipient Compatibility confirmed by FT-IR studies. All five phytosome formulations were incorporated with gel and formed clear, odorless, washable, homogeneous, stable and free from grittiness gel was evaluated under the various parameter, pH of all formulations was observed between 6.8 to 7.3 and Spreadability between 5.6 to 7.9 cm. % drug content between 98.9 % to 101%, and viscosity between 98 to 115 centi poice (cp) and % permeation between 83.2 % to 92.7 %. But on the basis of drug release kinetics **F-3** formulation was very good because its % drug release was **97.913%** followed **Higuchi** Kinetic Model. With the 95.56% drug release **F-2** formulation was good both follow First order

V. CONCLUSION

Neem leaves collected and extracted under soxhelate apparatus then physicochemical and phytochemical analysis was performd and characterize the drug .preformulation study was performed under the points as solubility studies, partition coefficient and drug excipient compatibility by FTIR and DSC analysis method.then after five different formulation of phytosomes was formulated and phytosomes were evaluated on various parameter like visuality, drug content, encapsulation efficiency and % drug release. Then all five types phytosomes were incorporated into gel and evaluated under gel parameter like clarity, griteeness, spredability, drug content, and diffusion study. Based on release kinetics formulation F-3 was very good formulation and F-2 was also good formulation.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

VI. REFERENCES

[1] Vaishali Jadhav, Vijaya Bhogale “Novel drug delivery system in herbal” International Journal of Pharma Wave. Vol. 1, Issue 2, 2015.

[2] Park K. Controlled drug delivery systems: Past forward and future back. J Control Release 2014;190:3-8.

[3] Sharma S, Sikarwar M. Phytosome: A Review. Planta Indica .2005; 1:1-3.

[4] Nilesh Jain, Brahma P Gupta, Navneet Thakur, Ruchi Jain, Jitendra Banweer, Deepak Kumar Jain, Surendra Jain “Phytosome: A Novel Drug Delivery System for Herbal Medicine” International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(4): 224-228.

[5] Yanyu X, Yunmei S, Zhipeng C, Quineng P. The Q preparation of Silybin phospholipidcomplex and the1 study on its pharmacokinetics in rats. Int J Pharm 2006; 307:77-82.

[6] Niyaz BB, Kalyani P, Divakar G: Formulation and evaluation of gel containing fluconazole-antifungal agent. International Journal of Drug Development and Research. 2011; 3(4): 109-128

[7] Goyal S, Sharma P, Ramchandani U, Shrivastava SK and Dubey PK: Novel antiinflammatory topical gels. International Journal of Pharmaceutical and Biological Archives. 2011; 2(4): 1087-1094.

[8] Nguyen Duc Hanh, Le Van Thinh, Do Quang Duong. Development and Evaluation of Neem Gel Formulation using Gum Karaya as Gelling Agent. Research J. Pharm. and Tech. 2020; 13(4): 1859-1864.

[9] Raju D, Jose J. Development and evaluation of novel topical gel of neem extract for the treatment of bacterial infections. J Cosmet Dermatol. 2019;18(6):1776-1783

[10] Narendra Ahirwal and Dr. S. K. Nayak. Formulation and evaluation of phytosomal gel of neem. World Journal of Pharmaceutical Research. 2021; 10(6):1177-1198.