METHODOLOGY

**Research approach**: Quantitative and Qualitative research approach

The main approach of our research is to utilize medicinal plant for elimination for physiological disorder in human. For this purpose we will prepare different types of extract and obtain chemical constituent along with glucosides. During experimental trial we consume different extract at different concentration along with their active constituents. We will try to achieve least amount of appropriate extract or active compounds instead of whole plant decognition and mixture of same compounds for elimination of physiological disorder.

**Research design:**

Pre experimental one group pretest & posttest design

We will design my research programme as under

1. Identification and collection of *Andrographis paniculata* from various places.
2. Extraction of solvent extract by soxhlet apparatus for 70-75 hours at controlled temperature.
3. Separation and Identification of compounds on the basis of TLC. We design our study by column chromatography for separation of all compounds will be purified by recrystallization and identified by CO-TLC, IR, $^1$HNMR, $^{13}$CNMR and mass spectroscopy.
4. Biological study

**Sampling technique :**

Non probability convenient sampling technique.

**Research tools :**

(1) For phytochemical study we utilize-
   Electric grinder, Oven, Sieve, Soxlet apparatus, Heating mantle, water bath, HPLC, UV, IR, NMR, Mass spectroscopy.

(2 ) For Biological study

(a). Albino rat, Guinea pig and Albino mice from equiped animal house.
(b). dissecting tray, homogenizer, Refregreted ultracentrifuse, Photocolorimeter, Laminar flow etc.

**Procedure:**
*Andrographis paniculata* plants will be obtained from field of Jaunpur and Ayodhya district Uttar Pradesh from various places. The aerial part of plant will wash thoroughly under running tap water and dried under shade. They will finally ground to a powder in an electric blender.

**(a) Extraction**

Petroleum ether (60:80) chloroform and ethyl alcohol will be used for plant extraction. With the help of soxhlet apparatus whole plant, leaf, stem and root powder exhaustively extracted with suitable solvent for 60-72 hrs separately. Each solvent extract obtained by distillation and calculate yield. Plant extract will preserved in cool at 4ºC temperature for phytochemical and biological study.

**(b) Separation of Compounds**

Plant extract will analysed for the presence of alkaloid, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoids and glycosides according to phytochemical methods. Thin layer chromatography chromatogram shows the presence of total compounds when process with solvent extract. To isolate andrographolide and other compounds in to pure form, we process column chromatography followed by crystallization. For purity of phyto constituents we will perform recrystallization of obtained compound following by TLC for single spot. The crude extracts will subject to TLC for developing solvent system using different ratios of hexane chloroform and ethanol. The TLC plate will expose to iodine vapour in a glass chamber for locating unsaturated compounds and Rf value will be calculate.

**(c) Identification of Compounds**

FTIR of isolated compounds will be process by model Vertex 70 Bruker, at the range 4000-400 cm\(^{-1}\). The various peaks in the spectra shows the presence of functional group in the isolated compound. With the help of spectral analysis UV, IR, \(^{1}\)HNMR, \(^{13}\)C NMR and Mass spectra we illucide the structure of isolated compounds. The spectral analysis will be done in analytical division, department of chemistry, Banaras Hindu University Varanasi U.P. We will prepare possible derivative of isolated compound. The identification and structure of derivative will be done by spectral analysis followed by co-TLC of authentic sample. To get pure form of compound we will perform recrystallization methods.
(d) Biological Study

*A. paniculata* is in demand in terms of its medicinal properties. It has been used for centuries in Asia to treat gastrointestinal tract, upper respiratory infections, fever, herpes, sore throat and a variety of other chronic and infectious disease. Plant powder, andrographolide use in different forms to prepare commercial drugs.

For biological study Albino rats (100-120 gm body weight) mice (80-90 gm body weight), guinea pig (18-200 gm, body weight.), pregnant rabbit (300-400 gm body weight) will be procured from animal house, Indian Institute of Medical Science, Banaras Hindu University Varanasi. These animals will be kept in normal room temperature and laboratory conditions. They will be kept normal water and animal protein diet for 15 days before start experiment. For each biological study required animals will be divided in four group as under :-

(i) **Control group:** Kept on normal water diet with drug vector during the experiment.

(ii) **Experimental group-1:** Kept on laboratory with ad libitum followed by minimum dose of drug with drug vector

(iii) **Experimental group-2:** Kept on laboratory ad libitum following middle dose of drug along with drug vector.

(iv) **Experimental group-3:** Kept on laboratory ad libitum following higher dose concentration of drug along with drug vector.

For each biological study, six animals will be select in each group. These animals will have administer drug for different duration considering that at least 2 to 3 animal fall in each similar category.

1. **Hepatoprotective effect:** We measure bile flow liver function test, liver enzyme. The protection of *A. paniculata* extract and andrographolide against CCl₄ and acetaminophen induced reduction in volume and hepatocyte content will be studied.
2. **Antimicrobial and antiparasitic effects**: This effect will be study against *Salmonella*, *Shigella*, group *A sterptococcus* and *Staphylococcus aureus* even at a concentration of 25 mg/mL crude powder. Growth parameter will select for this study.

3. **Cardiovascular effect**: We measure systolic and diastole blood pressure in hypertensive rat via pre-treated and post treated Albino rats. We will also measure angiotensin converting enzyme (ACE) lipid peroxidase enzyme to evaluate this effect.

4. **Antioxidant & Anti-inflammatory effect**: This experiment will be performed on Guinea pig 1% soln. of carcinogen produced inflammation of paw of guinea pig. We will calculate area of inflammation and biochemical enzyme responsible for this cause.

5. **Antifertility effect**: Pregnant rabbit will select for this study. We study causes of abortion at different gestation period in drug treated experimental animal.

6. **Glycemic effect**: Ethanol extract and andrographolide administered orally twice daily for 12-18 days to streptozotocin-induced rats and measure serum glucose, body weight SGOT and SGPT.

Phytochemical screening of *A. Paniculata* ethanolic extract revealed the presence of following functional groups of compounds.
Table 1: The analysis of phytochemical of the ethanolic extract of *A. Paniculata*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Phytochemical Test</th>
<th>Ethanol</th>
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<tbody>
<tr>
<td>1</td>
<td>Alkaloids - Meyer’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate &amp; glycosides – molisch’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Sugar – Benedict reagent Test</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Saponin – Foam Test</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td>Protein – Millon’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>Phytosterol – Liebermann Burchard’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic compounds &amp; Tannins – Ferric chloride Test</td>
<td>+ve</td>
</tr>
<tr>
<td>8</td>
<td>Flavonoids – Alkaline reagent Test</td>
<td>+ve</td>
</tr>
<tr>
<td>9</td>
<td>Glycosides – Legal’s Test</td>
<td>+ve</td>
</tr>
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Different phytochemicals have been found to possess a wide range of activities.

For antimicrobial study pure culture of *Escherichia, Staphylococcus aureus* species of bacteria and *Aspergillus niger, Aspergillus flavus* species of fungi leaf procured from microbiology laboratory Banaras Hindu University Varanasi. These organisms were identified and confirmed by professor D.D. Dubey Department of Biotechnology Institute of medical science Banaras Hindu University Varanasi, (U.P.)

Antibiogram was done by disc diffusion method using plan extract Petri plates were prepared by pouring 30 ml of NA/PDA medium for bacteria and fungi.

The test organism will be inert in inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes. The surface of media will be inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a evenly inoculated the entire surface of the nutrient agar/PDA plate. Briefly incolumns containing bacteria will spread on nutrient agar plates for bacteria and fungi sample spread on potato dextrose agar for fungus strains. Using sterile forceps the sterile filter papers (6 mm diameter) containing the crude extracts (50 µL, 100 µL and 150 µL) will be laid down on the surface of inoculated agar
plate. The plates are incubated at 37°C for 24 hour for the bacteria and at room temp (30±) for 24 to 48 hour for yeasts strains each sample will be tested in triplicate.

**Experimental Section:** All the melting part will be uncorrected. The U.V. spectra will determined on a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer. The I.R spectra will determined on a Perkin Elmer spectrum RX 1(4000 – 450 cm⁻¹). The ¹H NMR spectra will measured on a Bruker DRX-300 spectrometer using CDCl₃ as solvent and TMS as internal standard in ppm. The mass spectra will measured on Jeal SX-102 at 70 eV.

**Animals:** Animals will be used for pharmacological and biological studies are Albino rats, guinea pig and rabbit. Generally male albino rats weighing 100-120 gm will be procured for experiment. Animals were kept in individual cage in ventilated, humidity and temperature controlled room with a 12 hours light/dark cycle. They receive food pallets and water ad libitum. The local ethical committee for animal research approval all experiments.

Guinea pigs of either sex (300-400gm) will procured from the animal house. Department of kaya chikitsa institute of medical science BHU Varanasi U.P. India and will kept in standard plastic cages for two week acclimatization in the animal house unit of SRRM P.G. College jaunpur U.P. The animals will allowed free access to pellets (Hindustan laboratory India) and tap water they were exposed to natural light condition, room temperature and handle according to standard protocols for the use laboratory animals (National Institute of animal health 2002 India).

Rabbit of either sex (450-600 gm) will procured from the animal house, Department of pharmacology. IMS, Banaras Hindu University Varanasi India and will kept in standard plastic cages singly. Rabbit will allowed free access to pellets and tap water. Before start experiment they will acclimatize at least 15 days in animal house of laboratory.

**Preparation of plant extract:** Dry powder of *A. paniculata* whole plant leaf and root separately extracted for 70-80 hours with different solvent petroleum ether (60:80) Chloroform and ethyl alcohol by soxhlet apparatus. All plant extract will concentrate on water bath and preserve in refrigerator for phytochemical and biological study.
For experimental purpose, standard solution will be prepared for different experiment. For dose dependent experiment standard extract solution will be diluted as per requirement of experiment protocol. We will use normal saline as vector for dilution of standard extract for control group of experiment.
## WORK PLAN

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Tasks</th>
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| I YEAR | Completion of the coursework  
Review of literature  
Identification the problem  
Extraction of plant powder in different solvent  
Finalization of the research problem statement  
Formulation of the objectives, hypotheses and methodology |
| II YEAR | Tool preparation (Validity & Reliability) of the tool  
Obtain permission from the authorities and the subjects  
Data collection  
Analyzing the data and interpretation of study findings  
Summary preparation  
Submission of summary |
| III YEAR | Conference proceedings  
Paper publications  
Paper presentations  
Writing the thesis  
Submission of thesis |

*Andrographis paniculata* is an annual branched, erect and herbaceous plant which grows in hedgerows throughout the plane lands, hill slopes, waste ground, farms, moist habitat, seashores and road sides. Different habitat plants will be collected and authenticated by Dr. R.A. Singh, Department of Botany (Taxonomy) Kuteer P.G. College, Chakkey, Jaunpur (U.P.) 222002 India. Similar plant of authenticate sample will collect from at least three habitat and segregated in four part (a) whole plant (b) leaf (c) aerial part (stem) and root. All four parts will be dry in shade and powdery in light crusher.