CHAPTER – 2
MATERIALS & METHODS
This work embodies the result of plant materials for antidepressant activity study on *Camellia sinensis* Linn. The plants materials *C. sinensis* used for the present studies were commercially procured from local market of Indore, Madhya Pradesh, India.

*C. sinensis* (commonly known as Tea, Hindi – Chai). Tea plants are recognized as *Camellia sinensis* by botanists. They are small bushy plants about 3-4 feet high. Tea leaves are picked three to four times between spring and fall of each year. Green tea is prepared from leaves that are picked and heated quickly and are most often consumed in Western societies (Liao, *et al*., 2001). It has been generally believed for a long time in oriental cultures that tea has medicinal properties and being used in the treatment and prevention of diseases. According to Chinese history, about 47 centuries ago, Emperor Sheng-Nong found that many of the poisons of the body can be dissolve by drinking of a cup of tea, daily (Committee, 1991). Tea is antibacterial agents and being used as food preservative (Chung, *et al*., 1998).

There are nearly 4000 bioactive compounds present in the tea in which 1/3 presented as polyphenols (Tariq, *et al*., 2010). Other compounds are amino acids, carbohydrates, chlorophyll, alkaloidal (cafeine, theophyline & theobromine), fluoride, polypeptide, volatile organic chemicals, aluminum, minerals and trace elements (Cabirera, *et al*., 2003). Polyphinolic chemicals present in tea are mostly flavonoids (Sumpio, *et al*., 2006). The polyphenols, group contains catechins. The health benefits of the tea may be due to presence of flavonoids and catechins (Cabrera, *et al*., 2006).

Main cateichins are epicateichin galate (ECG), epicateichin (EC), epigalocateichin (EGC) and epigalocateichin gallate (EGCG). The catechin present in green tea is epigallocatechin – 3 – galate (EGCG), which is most active and abundant. Black tea contains relatively smaller contents of these catechins as compare to Green tea (Wu, *et al*., 2006). A combination of simple polyphenols, such as catechins and complex Polyphenols are reported in the Oolong tea (Mukhtar H. and Ahmad A., 2000).
Figure No. 9: Typical diagram of *Camellia sinensis* plant.
Figure No. 10: Picture of leaves of *Camellia sinensis* plant.
Figure No. 11: Picture of dry leaves of *Camellia sinensis* plant.
Figure No. 12: Typical picture of margins of leaf of *Camellia sinensis* plant.
Figure No. 13: Typical photograph of *Camellia sinensis* plant containing leaves, flowers and fruits.
Figure No. 14: A - Typical photograph of flowers of *Camellia sinensis* plant.
Figure No. 14: B - Typical photograph of flowers of *Camellia sinensis* plant.
Figure No. 15: Typical photograph of flowers and fruits of *Camellia sinensis* plant.
Figure No. 16: Typical photograph of flowers and fruits collected from the *Camellia sinensis* plant.
Figure No. 17: Typical photograph of fruit of *Camellia sinensis* plant.
Figure No. 18: Typical photograph of ripe fruit with seeds of *Camellia sinensis* plant.
Figure No. 19: Picture of fruits of *Camellia sinensis* plant with measurement.
Figure No. 20: Typical photograph of roots of *Camellia sinensis* plant.
1. Glassware

The glassware utilized for the purpose of extraction comprised of round bottom flasks of 2.5 liters capacity, soxhelete apparatus, weighing bottles and beakers. Before use the glasswares were dipped in chromic acid cleansing mixture diluted suitably with water and left for about 24 hours to soften any dry material sticking to the inner sides of the glassware, followed by very thorough washing with stiff jet of tap water. Then apparatus thoroughly brushed with detergent “Teepol” (Soap Solution) followed by an effective tap water wash and finally rinsed with distilled water. The glassware inverted and left to dry.

2. Extraction medium.

Solvents i.e. Pet. ether, CH\textsubscript{3}Cl, alcohol and aqueous solutions used in the extraction process. Chemicals used of analytical reagent grade.

3. Extraction method and conditions of leaves, fruits, marketed tea, flower and roots of *C. sinensis*

The leaves, fruits, marketed tea, flowers and roots dried in shade and then at 37°C. Leaves, fruits and roots cut into suitable size and reduced to coarse powder by grinder and passed through a sieve #10. The coarsely powdered of leaves, fruits, marketed tea, flower and roots (200 gm, each) extracted separately with petroleum ether (60-80°C), chloroform, ethanol (95 % v/v) successively using soxhlet apparatus till few drops of the last portion of the elute did not leave perceptible residue on drying. The ultimate dried mark of these three parts macerated with warm distilled water and filtered. Then the extractives obtained dried by evaporation of solvent under reduced pressure by ‘Rotavapour Apparatus’. Water extractives obtained by evaporation of water extract on hot plate in china dish. The extractives thus obtained from petroleum ether, chloroform, ethanol and water examined for their colour, phytoconstituents and antidepressant activities and results were noted.
Figure No. 21: Typical photograph herb grinding mill.
Figure No. 22: Typical photograph sieves.
Figure No. 23: Typical photograph of hating mantle.
Figure No. 24: Typical photograph of heating plate.
Figure No. 25: Typical photograph of Rotavapour apparatus.
4. Physical study of extracts

Different extractives of leaves, fruits, marketed tea, flowers and roots subjected to physical evaluation to detect their colours and chemical constituents. Phytochemical investigations were carried out as per the standard procedures mentioned in herbal pharmacopoeia.

5. Biological study

5.1 Animals

*Albino* mice (Laca strain) weighing 20-25 gm, breed Central Animal House of Pinnacle Biomedical Research Laboratories, Bhopal (Madhya Pradesh), India used for the study. The animals housed under standard 12 ± 1 hours light / dark cycle with food (Golden feed, New Delhi) and tap water *ad libitum*. The animals selected at random (male and female). The experiments conducted between 9.00 am to 5.00 pm.
Figure No. 26: Typical photograph of laboratory cage containing mice.
5.2 Drugs

The following extractives subjected to antidepressant studies:

**Leaves, fruits, marketed tea, flowers and roots extractives of *C. sinensis***

(i.) Petroleum ether extractives  
(ii.) Chloroform extractives  
(iii.) Ethanol extractives  
(iv.) Water extractives

5.3 Preparation of doses

Dried extractives suspended Tween 80 (2-5%) and then suspended in distilled water, to disperse the dose of the extractives and standard drug. Imipramine (Intas Pharmaceutical Limited, Ahmadabad) (10 m.g./kg) taken as the standard drug. All the drugs prepared afresh at the beginning of each experiment.
Figure No. 27: Typical photograph of weighting bottles for dose preparation.
5.4 Statistical analysis

Each experiment consisted of a group of minimum six animals. The data expressed as average immobility time ± Standard Error of Average. All the extractives have been compared with control and imipramine (standard) separately using one way analysis of variance (ANOVA) followed by Dunnett’s Method. Results at P<0.001 were considered statistically significant.

5.5 Animal model for antidepressant activity

Forced swim test (FST)
The mice were divided into 3 groups (n=Six). First group (control) which received Tween 80 suspended in distilled water (10 ml/kg) orally and second group which received reference drug 10 m.g./kg (orally) of Imipramine and third group which received extractives at 100, 200, 300 and 400 m.g./kg (orally). The FST was performed on mice by individually mice forced to swim in an open glass cylindrical jar (Height 25 cm and Diameter 10 cm), containing 15 cm of water at 25 ± 1°C. The total duration of immobility during the six minutes of test was recorded. Decrease in the duration of immobility during the FST taken as a measure of antidepressant activity (Porsolt, et al., 1977; Peng, et al., 2007).
Figure No. 28: Typical photograph of cylinders for forced swim test.
Figure No. 29: Typical photograph of stopwatch.

Figure No. 30: Typical photograph of thermometer.
Figure No. 31: Typical photograph of forced swim test.
Tail suspension test (TST)

The mice were divided into 3 groups (n=Six). First group (control) which received Tween 80 suspended in distilled water (10 ml/kg) orally and second group which received reference drug 10 m.g./kg (orally) of Imipramine and third group which received extractive at 100, 200, 300 and 400 m.g./kg (orally). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time recorded during a 6 minutes test (Steru, et al., 1985; Peng, et al., 2007).

The antidepressant activity of extractives and drug was evaluated by administering drug and extractives before 30 minutes of the evaluation of activity.
Figure No. 32: Typical photograph of tail suspension test.