## ANNEXURE – I

### List of publication

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Title of publication</th>
<th>Reference of publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>A study of antidepressant activity of marketed tea extracts of <em>Camellia sinensis</em>.</td>
<td>Under publication</td>
</tr>
</tbody>
</table>
A STUDY ON ANTIDEPRESANT ACTIVITY OF LEAVES EXTRACTS OF CAMELLIA SINENSIS

Marmat A*, Middha A.K.
Department of Pharmaceutical Science, JJT University, Jhunjhunu, Rajasthan

ABSTRACT:
Ethanol extract of leaves of Camellia sinensis have shown significant reduction in total immobility time in mice in forced swim test and tail suspension test at the different doses. However, antidepressant activity was as follows, ethanolic extracts > water extracts. Ethanolic extracts of leaves shown antidepressant activity was comparable to standard drug i.e. Imipramine (10 mg/kg). Tea plant contains more than 4000 bioactive compounds. The major parts of these compounds are flavonoids, polyphenols and catechins. Tea is reported to contain bioactive compounds of which one third is contributed by polyphenols. Polyphenols found in tea are mostly flavonoids. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits. The antidepressant activity of the deferent extract may be due to polyphenols (flavonoids and catechin) compounds.

Keywords: Camellia Sinensis, Tea, Antidepressant, Forced swim test, Tail suspension test, phytochemical investigation,

INTRODUCTION
C. Sinensis (commonly known as Tea, Hindi – Chai). Tea plants are recognized as Camellia sinensis by botanists. They are small bushy plants about 3 or 4 feet high. Tea leaves are picked three to four times between spring and fall of each year. According to Chinese history, about 47 centuries ago, Emperor Sheng-Nong reported that a daily cup of tea could dissolve many poisons in the body1. Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed by polyphenols2. Compounds are alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds, fluoride, aluminium, minerals and trace elements3. Polyphenols found in tea are mostly flavonoids4. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits that have traditionally been attributed to tea, especially green tea5. Major catechins are (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG). The most active and abundant catechin in green tea is epigallocatechin-3- gallate (EGCG)6. Oolong tea contains a mixture of simple polyphenols, such as catechins and complex Polyphenols7.

*Corresponding Author
Marmat A

Volume 3, Issue 5, September – October 2013
http://www.ijpi.org
In traditional Chinese and Indian medicine, practitioners used green tea to improving mental processes and health. Dating back more than 4,000 years, according to tradition, Chinese green tea could cure anything from headaches, body aches, and pains to constipation and depression.

Literature survey reveal that large number of activities shown by the plants (Camellia Sinensis) and their constituents. Anti-trypanosoma cruzi activity, Inhibition of liver enzyme, xanthine oxidase (XO), antibacterial activity, Antioxidant effects, Carcinogenicity, Antioxidant activity and radical scavenging capacity, Chemopreventive effects, Anti-inflammatory and antioxidant activity, Bactericidal effect, Antidiabetic activity, To suppress serum triglyceride, Anti-inflammatory, Analgesic and Antipyretic activities, Anti-ulcer activity, Anti-cholinesterase and β-secretase activities, Gastroprotective and hypoglycemic activities.

In the present study plant, Camellia Sinensis was evaluated for antidepressant activity. Literature shows that traditionally this plant is being use in the treatment of depression but no scientific and research data is available / reported to treat depression using this plant. Our attempt is to establish the scientific data of this plant as common, cheap and affordable, safe, effective, readily available alternative antidepressant agent.

Materials and Methods:
This research 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, India.

Extraction method and conditions of leaves of C. sinensis
The leaves were dried in shade and then at 37°C. Leaves were reduced to coarse powder by grinder and passed through a sieve #10. The coarsely powdered of leaves (200 gms) were 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 were macerated with warm distilled water and filtered. Then the extractives were obtained on evaporation of solvent under reduced pressure by ‘Rotavapour Apparatus’. Water extractives were obtained by evaporation of water extract on hot plate in china dish. The extractive thus obtained from petroleum ether, chloroform, ethanol and water were examined and their colour, phytochemicals and antidepressant activities were noted.

Photochemical investigations:
Phytochemical examinations were carried out as per the standard methods for extracts as mentioned below.

1. Detection of alkaloids: Extracts were dissolved in dilute Hydrochloric acid and filtered.

Mayer’s Test: Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner’s Test: Filtrates were treated with Wagner’s reagent (Iodine in Potassium
Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling’s A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins:
Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phytoesters:
Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

6. Detection of phenols:
Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. Detection of tannins:
Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was
added. Formation of white precipitate indicates the presence of tannins.

8. Detection of flavonoids:
   **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

   **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

9. Detection of proteins and amino acids:
   **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

   **Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10. Detection of diterpenes:
    **Copper acetate Test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

11. Detection of fixed oils and fats:
    A small quantity of petroleum ether or benzene extract is pressed between two filter paper. Oil stain on the paper indicates presence of fixed oils.
    Few drops of 0.5 N alcoholic potassium hydroxide is added to a small quantity of petroleum ether or benzene extract along with a drop of phenolphthalein. The mixture is heated on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fat.

12. Detection of volatile oils:
    About 50 gm of powdered drug material is taken in a volatile oil estimation apparatus and subjected to hydro distillation for the detection of volatile oil. The distillate is collected in the graduated tube of the assembly in which aqueous portion is automatically separated from the volatile oil, if it is present in the drug and returned back to the distillation flask.

**Evaluation of antidepressant activity**

**Animals:** Albino mice (Laca strain) weighing 20-25 gm, breed Central Animal House of Pinnacle Biomedical Research Laboratories, Bhopal (Madhya Pradesh), India were used. The animals were housed under standard 12 hours light / dark cycle with food (Golden feed, New Delhi) and tap water ad libitum the animals were selected at random (male and female). The experiments were conducted between 9.00 am to 5.00 pm.

**Drug treatment:** Petroleum ether extractives, Chloroform extractives, Ethanol extractives, Water extractives of leaves of C. sinensis were subjected to antidepressant studies. Dried extractives were suspended Tween 80 (2-5%) and then were suspended in distilled water, to disperse the dose of the extractives and standard drug. Imipramine (Intras Pharmaceutical Private Limited, Ahmadabad) (10 mg/kg) was taken as the standard drug. All the drugs were prepared afresh at the beginning of each experiment.

The drug, imipramine (10 mg/kg, orally) and various extractives (100, 200, 300 and 400 mg/kg) were administrated 30 minute prior to the experiment.
Statistical analysis: Each experiment consisted of a group of minimum six animals. The data is expressed as mean ± Standard Error of Mean. All the extractives were compared with control and imipramine (standard) separately using one way analysis of variance (ANOVA) followed by Dunnett’s Method. P<0.001 was considered statistically significant.

Animal model for antidepressant activity:
The Forssl swim test (PST) or forced swim test (FST) was developed as a rodent (mouse) screening test for human (potential) antidepressant drugs. This test is based on the assumption that an animal will try to escape a stressful (aversive) stimulus. If escape from the stressful situation is impossible, then animal eventually stops trying to escape and gives up. In the PST or FST, the mice are placed in a cylindrical container containing water from which they cannot escape. The forced swim test was carried out on mice individually forced to swim in an open cylindrical container (Diameter 10 cm, Height 25 cm), containing 15 cm of water at 25±1 °C. The total duration of immobility during the 5-min test was scored. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The duration of immobility was recorded. Decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity.  

The tail suspension test (TST) is based on the assumption that an animal will energetically try to escape a stressful (aversive) stimulus. If escape is impossible, the animal will finally stop trying (give up). In the Tail Suspension Test, a mouse is suspended by the tail, so that its body suspend in the air and rodent facing downward. 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 was recorded during a 6-min test. When the animal stops struggling and hangs itself immobile, it is considered to have “given up”. Longer period of immobility is characteristic of a depressive-like state.  

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1283/c/09/CPCSEA). Protocol Approval Reference No. was PBRI/12/IAEC/PN-335.

OBSERVATIONS AND RESULTS

Chemical study: The colours of extractives (Table - 1) and results of phytochemical investigations (Table - 2) were noted.

Antidepressant activity: The extractives of leaves of C. sinensis were subjected to forced swim test (FST) and tail suspension test (TST) for evaluation of antidepressant activity. Antidepressant effects of extracts were reported (Figure 1 to 8).
Table 1: Colour index of different extractives of *Camellia Sinensis*

<table>
<thead>
<tr>
<th>Leaves extractives</th>
<th>Colour of extractives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum Ether</td>
<td>Dark green</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Dark green to yellowish-brown</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Water</td>
<td>Brownish-black</td>
</tr>
</tbody>
</table>

Table 2: Results of Phytochemical evaluation of leaves extractive of *C. Sinensis*

<table>
<thead>
<tr>
<th></th>
<th>Petroleum Ether</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of alkaloids</td>
<td>---</td>
<td>✓</td>
<td>✓</td>
<td>---</td>
</tr>
<tr>
<td>Detection of carbohydrates</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>✓</td>
</tr>
<tr>
<td>Detection of glycosides</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Detection of saponins</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Detection of phytosterols</td>
<td>---</td>
<td>✓</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Detection of phenols</td>
<td>---</td>
<td>✓</td>
<td>✓</td>
<td>---</td>
</tr>
<tr>
<td>Detection of tannins</td>
<td>---</td>
<td>---</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Detection of Flavonoids</td>
<td>---</td>
<td>✓</td>
<td>✓</td>
<td>---</td>
</tr>
<tr>
<td>Detection of proteins and amino acids</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>✓</td>
</tr>
<tr>
<td>Detection of terpenes</td>
<td>---</td>
<td>---</td>
<td>✓</td>
<td>---</td>
</tr>
<tr>
<td>Detection of fixed oils and fats</td>
<td>✓</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Detection of volatile oils</td>
<td>✓</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
Antidepressant activity of different leaves extracts of C. Sinensis in FST Model

Figure - 1, Antidepressant activity of petroleum ether extracts of leaves of C. Sinensis in FST Model

Figure - 2, Antidepressant activity of chloroform extracts of leaves of C. Sinensis in FST Model

Figure - 3, Antidepressant activity of ethanol extracts of leaves of C. Sinensis in FST Model

Figure - 4, Antidepressant activity of water extracts of leaves of C. Sinensis in FST Model
Antidepressant activity of different leaves extracts of *C. Sinensis* in TST Model

![Graph 1: Antidepressant activity of leave extracts of Camellia Sinensis](image1)

Figure - 5, Antidepressant activity of petroleum ether extracts of leaves of *C. Sinensis* in TST Model

![Graph 2: Antidepressant activity of leave extracts of Camellia Sinensis](image2)

Figure - 6, Antidepressant activity of chloroform extracts of leaves of *C. Sinensis* in TST Model

![Graph 3: Antidepressant activity of leave extracts of Camellia Sinensis](image3)

Figure - 7, Antidepressant activity of ethanol extracts of leaves of *C. Sinensis* in TST Model

![Graph 4: Antidepressant activity of leave extracts of Camellia Sinensis](image4)

Figure - 8, Antidepressant activity of water extracts of leaves of *C. Sinensis* in TST Model
Summary and conclusions:
Ethanol extracts of leaves have shown significant reduction in total immobility time in mice in both the animal models at the all doses. However, antidepressant activity was as follows, ethanolic extracts > water extracts. Ethanolic extracts of leaves shown antidepressant activity was comparable to standard drug i.e. Imipramine (10 mg/kg).

As reported earlier, tea plant contains more than 4000 bioactive compounds. The major parts of these compounds are flavonoids, polyphenols, and catechins. All these are the biologically active compounds. Tea is reported to contain bioactive compounds of which one third is contributed by polyphenols. Other compounds are alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds, fluoride, aluminium, minerals and trace elements. Polyphenols found in tea are mostly flavonoids. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits that have traditionally been attributed to tea, especially green tea. Major catechins are epicatechin gallate, epicatechin, epigallocatechin, and epigallocatechin gallate. The most active and abundant catechin in green tea is epigallocatechin-3-gallate. The antidepressant activity of the different extract may be due to polyphenols (Flavonoids and catechin) compounds.

REFERENCE:
10. Cristina P. Stanc C., Monica IE., Virginia M., Jorge C., Mirkha MF., Hector NT. Anti-Trypanosoma


17. Kapadia GJ., Paul BD., Chung EB., Ghosh B., Pradhan SN. Carcinogenicity of Camellia sinensis (tea) and some tannin-containing folk medicinal herbs administered subcutaneously in rats. Journal of the National Cancer Institute. 1976; 57(1), 207-209.


22. Gomes A, Vedasiromoni JR., Das M., Sharma RM., Ganguly DK. Anti-hyperglycemic effect of black...


34. Peng WH., Lo KL., Lee YH., Hung TH., Lin YC. Berberine produces antidepressant-like effects in the forced swim test and in the tail


CERTIFICATE

TO WHOM SO EVER IT MAY CONCERN

This is certified that the experimental work 'A STUDY ON ANTIDEPRESSANT ACTIVITY OF EXTRACTS OF CAMELLIA SINENSIS' was carried out in our lab. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Registration No. 1283/c/09/CPCSEA).

The study protocol approval reference no. is PBRI/13/IAEC/PN-335.

Bhopal

[Signature]

Scientist

Pinnacle Biomedical Research Institute
BHOPAL (M.P.)