

Study on Antidepressant Activity of Various Extracts of *Camellia Sinensis*

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Abstract: *This study evaluated the antidepressant activity of aqueous, ethanolic, and methanolic extracts of Camellia sinensis leaves in Swiss albino mice using Forced Swim Test and Tail Suspension Test. Methanolic extract at 400 mg/kg showed the highest activity, significantly reducing immobility time comparable to imipramine, with no CNS stimulant effect. The activity correlated with higher phenolic and flavonoid content. Results suggest methanolic extract of C. sinensis has potent antidepressant-like potential and may serve as a source for plant-based antidepressant development.*

Depression is a prevalent neuropsychiatric disorder with significant morbidity. Conventional antidepressants exhibit delayed therapeutic onset, incomplete efficacy, and adverse effects, prompting investigation into safer plant-derived alternatives.

Camellia sinensis, commonly known as tea plant, is one of the most widely consumed medicinal plants in the world. Green tea leaves contain biologically active compounds such as catechins, polyphenols, flavonoids, alkaloids, caffeine, epigallocatechin gallate (EGCG), and L-theanine. These phytoconstituents possess antioxidant, neuroprotective, anxiolytic, anti-inflammatory, and antidepressant properties.

The results showed significant antidepressant activity in all extracts compared to control groups. Among the tested extracts, methanolic extract exhibited maximum antidepressant activity, followed by ethanolic extract. Reduction in immobility time in both FST and TST indicated significant antidepressant activity. The study concludes that Camellia sinensis possesses promising antidepressant activity and can serve as a potential herbal alternative for.

Keywords: Camellia sinensis, Anti-depressant, FST, TST, methanolic extract

I. INTRODUCTION

Tea polyphenols are one of the main components in the formation of the color and flavor of tea soup and are also important ingredients for tea with health functions. The species, processing method, and fermentation degree are the key factors that affect the content of tea polyphenols in tea. Gao et al. analyzed the content of 16 common tea leaves and found that the content of tea polyphenols in green tea was the highest. They suggested that green tea was the preferred tea source for the development of tea polyphenol functional foods. In recent years, numerous domestic and foreign studies are focused on the chemical composition and pharmacological effects of green tea. At present, there is a lack of systematic and comprehensive review on the research results of green tea. In this paper.

A large number of researchers have confirmed that green tea possesses chemical ingredients that are closely related to human health. Tea polyphenols, caffeine, theanine, tea polysaccharides, and other components which are extracted and separated from green tea have pharmacological activities such as anti-cancer [3], anti-oxidation [4], protecting the nervous system [5], and lowering blood sugar [6]. Green tea has been considered to be suitable for patients with hypertension, hyperlipidemia, coronary heart disease, arteriosclerosis, and diabetes. However, it is important to keep in mind that "natural" does not mean perfectly safe.

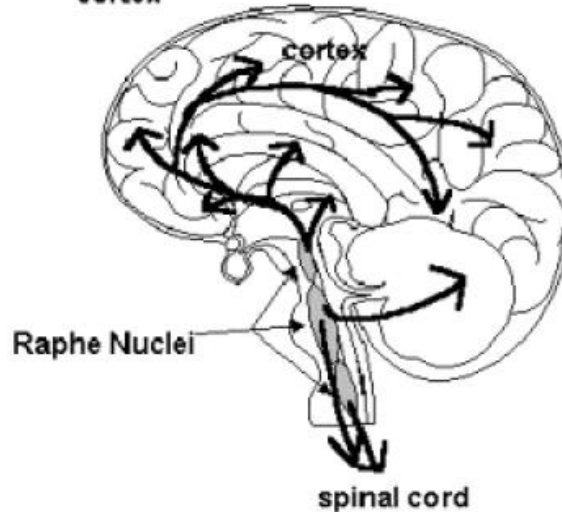
Although the toxic side effects of green tea are relatively small, it must be used with caution in pregnancy, children, and the elderly population. Tea polyphenols are one of the main components in the formation of the color and flavor of tea soup.



Depression: It is basically acknowledged as illness with symptoms such as anxiety and sleep disturbances (Stahl S.M. 2000). It can be a persistent, recurring illness that can cause many personal suffering for individuals and their families. At present, disability caused by depression is estimated to be the fourth most important cause of worldwide loss of life years (Mulrow C.D., et al.,1998).

Serotonin projections

Raphe Projects throughout the cortex



No. 1: The projections of the serotonin system
Locus ceruleus projections

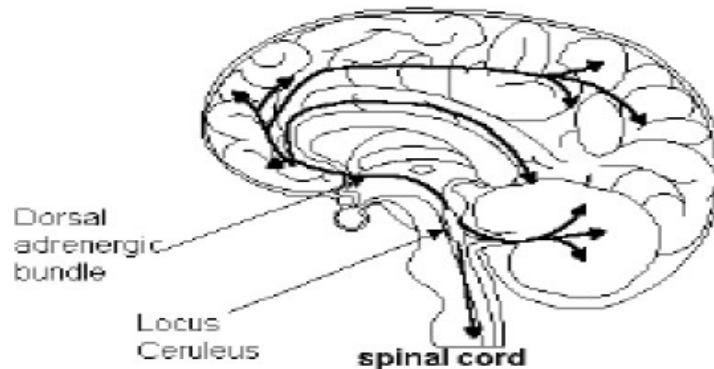


Fig. No. 2: The projection of the noradrenaline system

The nuclei of the dorsal raphe projects the serotonin system and the raphe Magnús. The serotonin receptors (5-HT) have been identified into various sub-types with the 5-HT1 and 5-HT2 sub-types being of greater interest in psychiatry. The most important of the 5-HT1 subclass is 5-HT1A which is this has not been shown to influence the efficacy of the drug (Waller D.G., et al., 2001). The different monoamine reuptake properties can also include an increase in dopaminergic activity via a presynaptic mechanism for amitriptyline and a post synaptic mechanism for desipramine and imipramine (Besson A., et al., 1999).



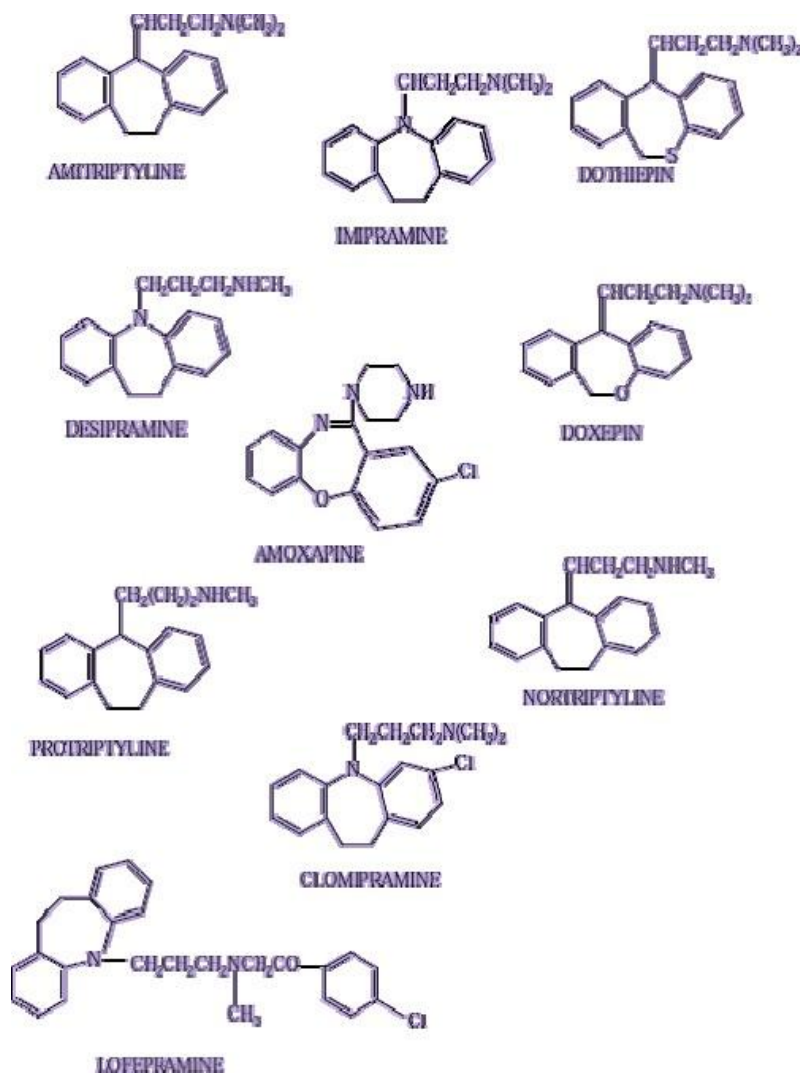


Fig No. 3: Chemical structures of some tricyclic antidepressants

B. Heterocyclics

Between 1980 and 1996 “heterocyclic” antidepressants were discovered. Examples of these can be seen in Figure No. 4 shown below. Amoxapine and maprotiline resemble the structure of the TCAs while trazadone is distinctly different. Maprotiline is similar to the TCA, desipramine in being a potent noradrenaline reuptake inhibitor and it has less sedative and antimuscarinic side effects. Amoxapine is a metabolite the antipsychotic drug oxepine and displays some dopamine receptor antagonism. Trazadone has shown unpredictable efficacy in the clinical setting (Potter W.Z. and Hollister L.E., 1998).



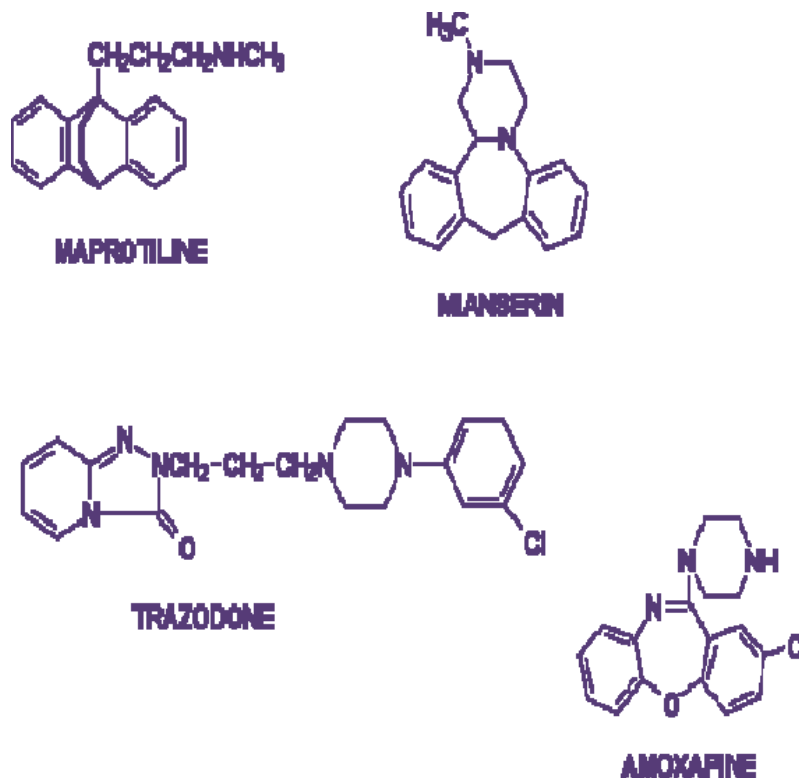


Fig No. 4 Chemical Structures of Some Heterocyclic Antidepressants

C. Selective Serotonin Reuptake Inhibitors (SSRI)

Unlike the tricyclic antidepressants, the SSRIs reduce the neuronal uptake of serotonin but have no effect on noradrenaline. Therefore the SSRIs have a better side effect profile in comparison with TCAs because these drugs have a low affinity for muscarinic, histaminergic and adrenergic receptors (Waller D.G., et al., 2001).

Fluoxetine was the first SSRI to be used clinically followed by paroxetine and sertraline. The latter two have shorter half lives and do not inhibit specific P450 isoenzymes (Potter W.Z. and Hollister L.E., 1998). The chemical structures of these SSRIs are shown in Figure No. 5 below.



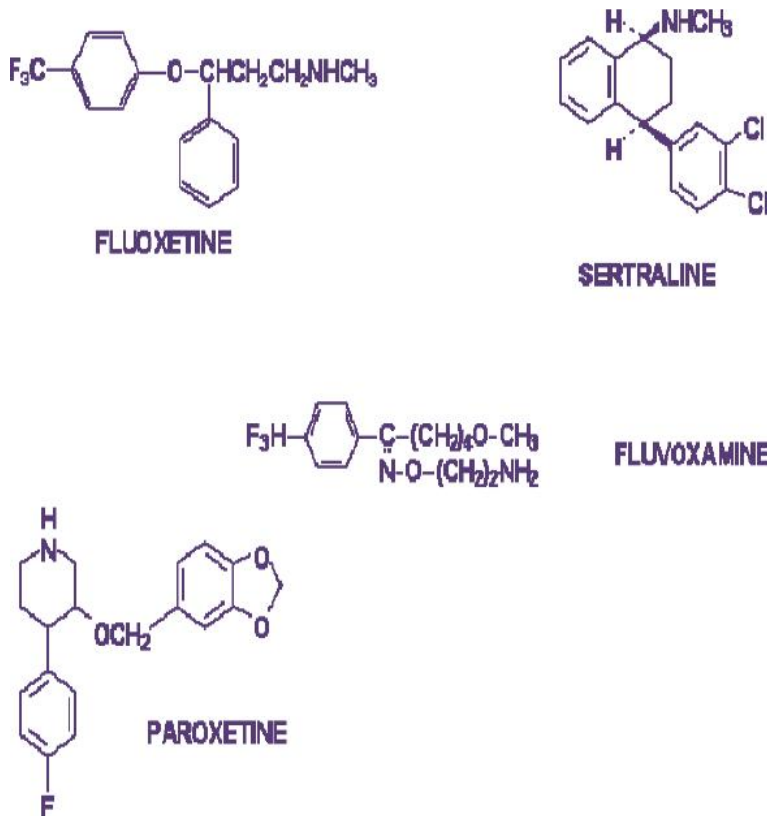


Fig No. 5: Chemical Structures of Some SSRI Antidepressants

2. PATHOPHYSIOLOGY

Biogenic amine hypothesis

The primary path physiologic abnormality in depression may occur due to an

Review of literature

1. Singh R.K., et al., (1998)

Reported that Benzene (BE), Petroleum ether (PE), Acetone (AE), ethanolic (EE), Chloroform (CE) extractive obtained from dried leaves of *Abies Pindrop* showed significant antidepressant activity in rats when given 30-45 min before. Chemically extract showed the presence of glycoside, steroid, terpenoids and flavonoids. It has been reported that ethanolic extract of *A. Pindrop* having glucopyranoside, hydroxy-flavone and chalone glycoside, bioflavonoids.

2. Hasrat J.A., et. Al., (1997)

Found that the fruits and leaves extract of *Anona muricata* inhibit binding of [3H] rauwolscine to 5-Serotonergic 5-HT_{1A} receptor in *Calf hippocampus* & showed antidepressant activity. The three alkaloids (is quinoline derivative) annonacin, nonuniformed and assimilating isolated from the extracts of fruits.

3. Hoong D.T.L., et al., (2002)

Fractionated and isolated of dichloromethane fraction of *Aquilaria agalloch* by bioassay-directed fractionation. Four compounds having MOA inhibitory effect were isolated by repeated silica gel column chromatography.



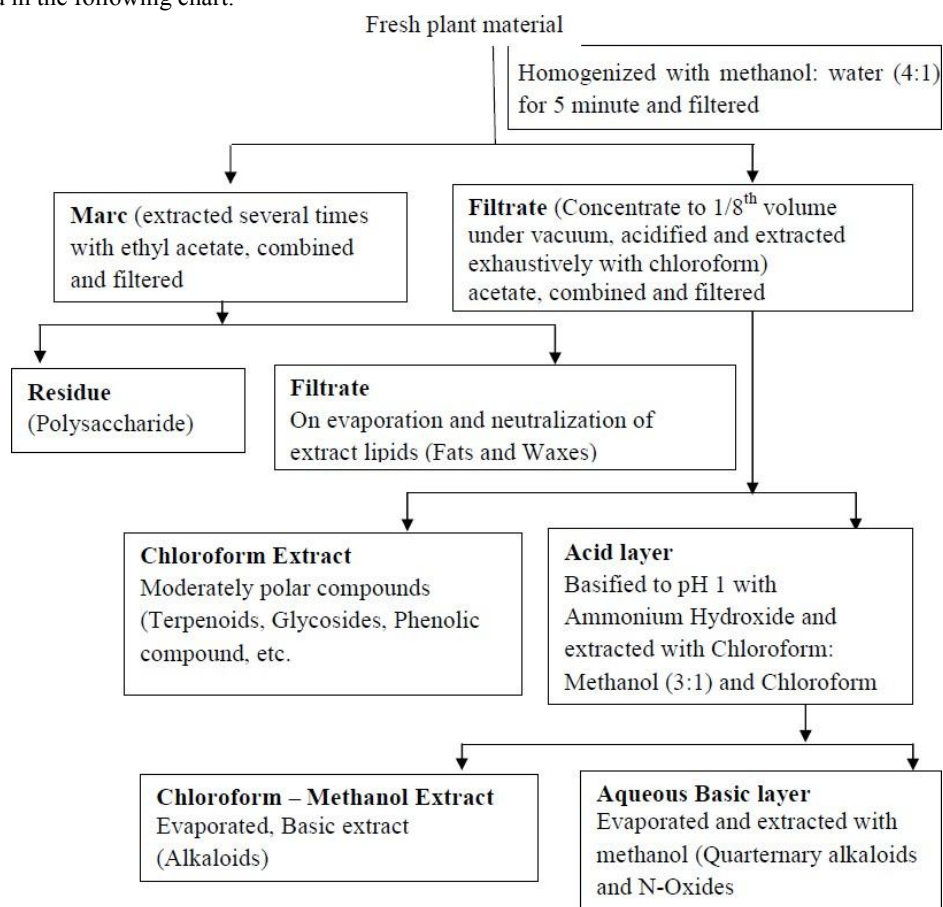
These structures were established as psoralen, 17ox let17a, alpha amyryn acetate and 5-hydroxymethylfurfural with the help of their physiochemical and spectral data. Among these compounds psoralen and 17ox let17a showed high inhibitory activities in vitro against mouse brain monoamine oxidase hence proved as antidepressant.

4. Nishibe S., et al., (2002)

The powdered plant is extracted by 250x let apparatus using different grade of solvents from n-hexane, n-heptane, DMF, CCl₄, ethyl acetate, alcohols, water & Acetic acid in increasing polarity.

The concentrated extract is generally obtained by distillation of the solvent under low pressure followed by evaporation until dryness. The extracts with different solvents can also be prepared by successively maceration (cold extraction) of the powdered drug in order of increase polarity.

The general approach for extraction of different constituents from the freshly plant material may be briefly described in the described in the following chart.



It is quite obvious that the extract of phytoconstituents prepared by maceration or percolation method must be as pure as possible and unless it is reasonably so, the test reaction may not be accurate. Therefore, some purification procedures are usually adopted prior to characterization of individual components.

There is always necessitates further purification of plant extracts, which can be performed by various techniques like fractional crystallization, fraction liberation, sublimation, distillation, etc.

(Kok ate K.C., et al., 1999).





Fig.6 Schematic diagram Soxhlet Apparatus



Fig No. 7: Representative diagram for Forced Swim Test



Tail-suspension test (TST) in mouse (rat) was developed for human as a potential screening test for antidepressant drugs. It is a stressful animal situation based on the assumption that the animal will try to escape this stressful situation. It is impossible to escape, finally animal stopped trying to escape (give-up). In the tail suspension test, Mouse or Rat hanging in the air and their body faces downward. The test period is six or more minutes. The test can be repeated several times. At the beginning, rat will try to climb on a solid surface.

The animal struggles to escape from this situation after that stops and finally give up. Long periods of immobility indicates a depressive state. The verification of the validity of treatment with an antidepressant medication decreases the immobility time of the animal (Thierry B., et al., 1986).

Following considerations should be considered during the TST are:

1. Mice should be suspended above the “cushioned” surface, to help prevent injury to the mice if it falls. Mice that may experienced a fall should be removed from the experiment (Bergner, et al., 2010).
2. Vinyl or medical adhesive tapes is recommended for the hanging of mice adhesive tape should be applied in a consistent position $\frac{3}{4}$ of the distance from the base of the mice’s tail (Porzelt, et al., 2009).
3. Some strains (for example, C57BL/6J) may not perform very well in TST, to tail climbing behaviour. These types of mice should not be used in the TST. (Bergner, et al., 2010).

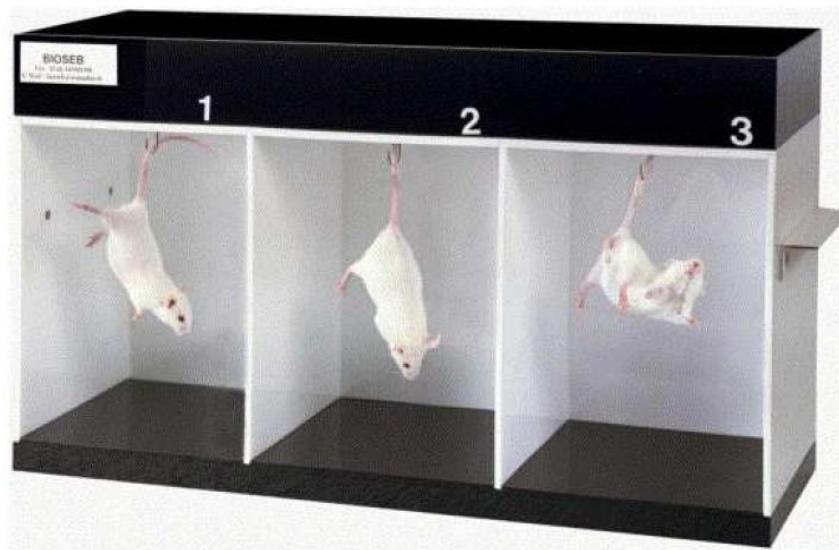


Fig No. 8: Representative diagram for Tail Suspension Test

STATEMENT OF THE PROBLEM

As per world Health Organization (WHO), worldwide, depression is a common illness, it was estimated that 350 million people are affected from this illness. Everyday life’s mood fluctuations i.e. usual and short-lived emotional

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 o 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 (caffeine, theophylline & theobromine), fluoride, polypeptide, volatile organic chemicals, aluminium, minerals and trace elements (Cabrera, e.t al., 2003). Polyphinolic chemicals present in tea are mostly flavonoids (Sumption, 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 catechins are epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). The catechin present in green tea is epigallocatechin – 3 – gallate (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).



Fig No. 9: Typical diagram of Camellia sinensis plant.





Fig No. 10: Picture of leaves of *Camellia sinensis* plant. Identification Features

- Leaves: Smooth, shiny, dark green, and oval-shaped
- Edges: Small serrations (toothed margins)
- Texture: Thick and slightly leathery
- Growth: Leaves grow alternately on woody stems

This plant is one of the world's most important beverage crops. Young leaves and buds are harvested for making tea.

Tea from these leaves contains:

- Antioxidants
- Caffeine
- Polyphenols

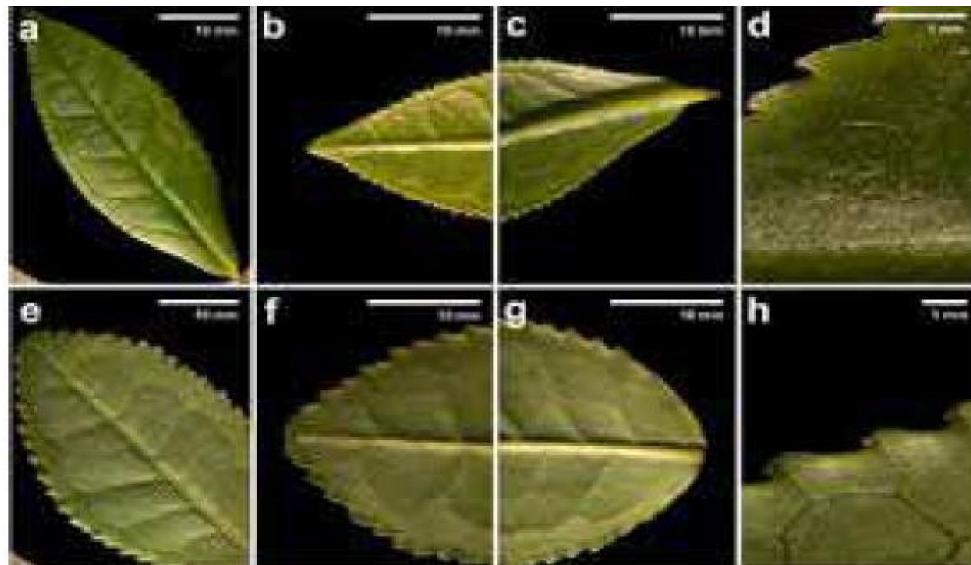


Fig No. 11: Typical picture of margins of leaf of *Camellia sinensis* plant



The image shows a comparative study of leaves from the *Camellia sinensis* under different magnifications and views. The panels labeled a–h display the morphology (shape and structure) and surface characteristics of tea leaves, likely observed for botanical or scientific analysis. The upper panels (a–d) show smoother and narrower leaves, while the lower panels (e–h) show broader leaves with more visible serrated edges and surface textures. These differences may represent variations between tea cultivars, leaf maturity stages, or environmental adaptations. Tea leaves are generally lance-shaped or elliptical with pointed tips, a central midrib, and fine serrations along the margins. The glossy green appearance indicates healthy chlorophyll-rich tissue important for photosynthesis and tea quality.



Fig No.12: Typical photograph of flowers and fruits of *Camellia sinensis* plant

The image shows a close-up view of a flowering plant branch with a beautiful white flower, green fruits, and glossy dark green leaves. The flower has soft white petals arranged in a circular pattern with a bright yellow cluster of stamens in the center, making it visually attractive and fresh. Alongside the flower, two round green fruits or buds are visible, indicating different stages of the plant's growth and development. The stem appears woody and brown, supporting the flower and fruits. The shiny leaves surrounding the branch suggest that the plant is healthy and evergreen. This type of image is commonly used in botany and biology studies to explain the reproductive parts of flowering plants, including petals, stamens, buds, fruits, and leaves. The contrast between the white flower, yellow center, and green fruits creates a natural and vibrant appearance, highlighting the beauty and life cycle of the plant.





Fig No. 13: Typical photograph of ripe fruit with seeds of *Camellia sinensis*

The image shows a mature fruit of a flowering plant that has naturally split open to reveal its seeds inside. The outer covering of the fruit is brown, rough, and hard, indicating that it is fully ripened. As the fruit opens, shiny dark brown to black seeds can be seen arranged around a pale white central structure. The split fruit demonstrates the process of seed dispersal, where mature fruits open to release seeds for reproduction. Surrounding the fruit are smooth, elongated green leaves that provide a healthy and natural background. The image is useful for studying plant morphology and reproductive biology because it clearly displays the structure of a mature fruit, including the fruit wall, seeds, and internal arrangement. The contrast between the brown fruit shell, dark seeds, and green leaves creates an attractive natural appearance and highlights the final stage of the plant's reproductive cycle.





Fig No. 14: Picture of fruits of *Camellia sinensis* plant with measurement.

The image shows several small, round to oval-shaped brown seeds placed beside a measuring scale for size comparison. The seeds appear hard, smooth, and dark brown in color, with a slightly glossy outer surface. The ruler in the background indicates that the seeds are approximately 1 to 2 centimeters in size. Their tough outer covering protects the inner embryo and stored food material, which are essential for germination and the growth of a new plant. The arrangement of the seeds on a plain surface makes it easier to observe their shape, texture, and dimensions clearly. Such images are commonly used in botany, agriculture, and plant taxonomy studies to identify plant species based on seed characteristics. The photograph highlights the importance of seeds in plant reproduction, as they contain the genetic material necessary for producing the next generation of plants.





Fig No. 15: Typical photograph of roots of *Camellia sinensis* plant

Instruments

1. Glassware

The glassware utilized for the purpose of extraction comprised of round bottom flasks of 2.5 liters capacity, Soxhlet 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.



Fig No. 16: Typical photograph herb grinding mill.

The image shows a mechanical industrial machine commonly used for crushing, grinding, or pulverizing agricultural and food materials such as grains, spices, seeds, herbs, or other solid substances. The machine consists of a strong



metal frame painted in blue, which supports the entire structure and provides stability during operation. At the top, there is a hopper or feeding chamber where raw materials are inserted into the machine. The central metallic chamber contains the grinding or crushing mechanism that processes the material into smaller particles or powder. A powerful electric motor is attached at the lower side of the machine.



Fig No. 17: Typical photograph of Rotavapor apparatus.

3. 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.

4. Biological study 4.1 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.



Fig No. 18: Typical photograph of laboratory cage containing mice.



4.2 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

4.3 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



Fig No. 19: Typical photograph of weighing bottles for dose preparation.

4.4 Statistical analysis

Each experiment consisted of a group of minimum six animals. The data expressed as average immobility time \pm 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.

4.5 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 $25 \pm 1^\circ\text{C}$. 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).





Fig No. 20: Typical photograph of cylinders for forced swim test.

The image shows two transparent cylindrical glass containers placed side by side on a plain background. One container is taller and narrower, while the other is shorter with a slightly wider appearance. Both are made of clear glass with smooth surfaces and rounded bases, giving them a simple and elegant design. These types of glass vessels are commonly used in laboratories, homes, hotels, restaurants, and decorative settings. In laboratories, such cylindrical containers may be used for storing liquids, conducting experiments, or measuring substances.

II. CONCLUSION

In this paper, the phytochemical constituents and pharmacological activities of green tea were systematically and comprehensively reviewed. Catechin, caffeine, theanine, tea polysaccharide, and other chemical components in green tea have pharmacological activities and health care functions, such as antioxidant, anti-tumor, hypoglycemic, and so on. As a natural antioxidant, tea polyphenols have been widely used in the food industry and cosmetics. In addition, the catechins in green tea also play an important role in the prevention and treatment of diabetes, hepatitis, microbial/viral infections, cancer, and skin inflammation.

In 2006, the FDA approved “Veregen ointment”, a green tea extract external preparation, for clinical use and it has already appeared on the market in the United States. However, the research on the pharmacological activity of green tea is still in the laboratory research stage. Therefore, how to carry out in-depth research on the mechanism of green tea active ingredients and realize the conversion from research to clinical application is still a major challenge facing researchers. Finally, there are few reports on toxicological studies of green tea, mainly related to hepatotoxicity and cytotoxicity. Therefore, toxicity studies are still a potential research area in the future. Furukawa et al. investigated whether EGCG observed that EGCG promoted the formation of 8-oxide, a characteristic oxidative damage could cause oxidative damage to in vitro bovine thymus DNA under the action of metal ions and H₂O₂ oxidative stress. They found that DNA is strongly associated with mutations and cancer [128]. Therefore, they came to the conclusion that this oxidative damage to EGCG could be considered as a potential predisposing factor for EGCG carcinogenicity [129]. One study showed that EGCG (20, 40 and 80 μ M, 10, 60, and 240 min) caused DNA damage in both human lymphocytes and Nalm6 cells in a dose-dependent manner. When the maximum dose of EGCG was 100 μ M, the survival rate of Nalm6 and human Lymphocyte decreased by 50% and 25%, respectively.



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