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# ESTABLISHMENT OF PHARMACOGNOSTIC STANDARDS OF DIFFERENT MORPHOLOGICAL PARTS OF *CAMELLIA SINENSIS* L. GROWN IN PAKISTAN

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**Abstract**

*Camellia sinensis* L. is well known plant, belongs to the family Theaceae. In the present investigation, the pharmacognostic standardization of stem leaf and seed was carried out. Macroscopic, microscopic, preliminary phytochemical screening and fluorescence analysis of different morphological parts of C. *sinensis* L. were carried out. The aim of present study is not only to evaluate novel and attractive features of such an important plant but also to elaborate the quality of this common plant which is considered most popular drink in this region. The macroscopic and microscopic evaluation showing the analytical and diagnostic features of different morphological parts of the plants by sensory method is helpful for the authentication of the plant. Phytochemical screening assists in determining the primary and secondary metabolites like flavonoids, resins, tannins, alkaloids, glycosides, fixed oils, volatile oils, lignins, saponins, steroids, terpenes, carbohydrates, proteins, fats and amino acids. The investigation was made by the fluorescence analysis at both visible and UV light (i.e, 254 and 366λ max) which was not only useful in detecting the colored compounds visible to naked eye but also helpful in gaining the information about the chromophore group and conjugation system. It indicated that the purity and quality of the plant drug could be established by pharmacognostic evaluation method. The results shows the presence of primary metabolites in different quantity in various plant parts which are helpful for identification, therapeutic and pharmacological evaluation and even for the development of monograph as the food cum drug.

**Key words:** *Camellia sinensis* L., Pharmacognostic, Phytochemical, Fluorescence analysis, Powder microscopy.

## Introduction

Herbal medicines are derived from the plants directly or can be delivered in various forms like solid, liquid, inhalant, or in topical form for direct application on the skin. Mostly root, seeds, leaves, barks and flowers are used for herbal preparation. Herbal drugs either obtained the whole from plant or plant parts are converted into phytopharmaceuticals by simple processes like harvesting, drying and storage of crude drugs (Ijaz *et al.*, 2021). Traditional medicine is considered an important element of the culture. The western world is attracted to herbal treatment for several reasons, mostly because our forefather believed that they could assist us in animate better lives and their reasonable price and modest approach towards healing. People spend billions of dollars on herbal medicines and use them as home remedies and over the counter drugs (Folashade *et al*., 2012; Iqbal *et al*., 2020). The advancement in phytochemical studies has gained the attention of phytochemists due to the development of new techniques. These methods are crucial in the hunt for additional raw material sources for pharmaceutical sector (phytochemicals) (Mungole *et al.*, 2010). Alkaloids, phenols, flavonoids, saponins, terpenoids and tannins etc., are the secondary metabolites present in minor quantities in higher plants that can serve as phytochemicals (Peteros & Uy, 2010; Rubab *et al*., 2017). World Health Organization (WHO) states that 80% of the world population is dependent on herbal medicine for their healthy living (Hassan & Ullah, 2019). Approximately 450,000 angiosperm species of the plants are present on the earth, out of which 10 to 20% are still unknown. Among these 6% plants have been screened for therapeutic significance and 15% are evaluated phytochemically (Turker & Usta, 2008; Corlett, 2016). Tea plant, is an evergreen shrub or small tree, *C. sinensis* L. belongs to the family Theaceae (Lu *et al.*, 2012; Rubab *et al*., 2020a) having different varieties. There are three different taxa which are recognized by commercial tea cultivators, *C. sinensis, C. assamica,* and *C. assamica* ssp. *lasiocalyx* conversely, tea is extremely diverse and all taxa freely inter-breed (Gulati *et al.*, 2009). Tea plant, tea tree and tea shrub are its common name (Nikam *et al.*, 2012). Pakistan is considered as the second highest among the tea importer countries. Tea is one of the most preferred drinks in Pakistan (Latif *et al.*, 2008; Rubab *et al*., 2020b).

The evergreen shrub of the tea plant endows with black, white, yellow, and green tea as well as cooling and power tea. The leaves and leaf buds are mostly used to make tea. The chief flavonoids found in the green tea consist of catechins (flavin-3-ols). There are four types of catechins present in the green tea including epigallocatechin-3-gallate (EGCG) which constitute about 59% of entire catechins along with approximately 19% of epigallocatechin (EGC); almost 13.6% of epicatechin-3-gallate(ECG); and about 6.4% of epicatechin (EC) (McKay & Blumberg, 2002). In addition to catechin green tea also contains gallic acid (GA) and other phenolic acids like chlorogenic acid and caffeic acid. Besides flavonols i.e., myricetin, kaemferol and quercetin are also present. Most of the studies have revealed the useful effects of green tea consumption on kidney stones, bone density and cognitive functions despite other effects (McKay & Blumberg, 2002; Wu & Wei, 2002). The traditional Chinese medicine believed that tea plant could be useful in the treatment of headache, pain, body aches, digestion, and detoxification, as a refresher and life protector. Green tea contains mainly four types of the components which affect the human life that are essential oils, caffeine, theophyllin (xanthic bases) and polyphenolic compounds. Central nervous system is affected by the caffeine which stimulates alertness and decreasing the feeling of exhaustion (Varnam & Sutherland, 1994). The purpose of the study is to evaluate the phytochemical importance of *C. sinensis* L. which is most common beverage in Pakistan.

## Materials and Methods

Samples of *C. sinensis* L. were collected for this research from National Tea and High Value Crops Research Institute (NTHRI) in the month of March 2016. After identification the voucher specimen with numbers 0121a, 0121b, 0121c and 0121d were deposited in the pharmacognosy museum, Department of Pharmacognosy, University of the Karachi. Samples were then kept in shade for 15 days. When all the parts of plant were sufficiently dried, each part was pulverized separately to a fine powder form and each powdered sample was passed through sieve # 120, stored in amber coloured bottles, preserved at ambient temperature and pressure conditions. For extraction, 50gram powder of each component of the plant (root, stem, leaf, and seed) was soaked separately in 500mL of methanol of analytical grade (Merck) in glass aspirators for seven days with occasional shaking at room temperature. Filtration was carried out using Whatman filter No 1. Rotary vacuum evaporator (Rota vapour R-200, Buchi) was used for evaporation of filtrate at temperature 50°C with rotation 40rpm and pressure 0.07MPA or 20 in Hg. The dried material was weighed, labelled and stored in the refrigerator at 4°C. These condensed extracts were then used for further screening of phytochemical analysis.

**Macroscopic analysis:** Macroscopic evaluation of root, stem, leaf and seed were carried out. The taxonomical description was noted as mentioned by (Ishtiaq *et al.*, 2018a). Texture, color taste and odour of all morphological parts of *C. sinensis* L. were noted (Patil *et al.*, 2013; Aslam & Afridi, 2017).

**Phytochemical investigations:** For preliminary screening and identification of major constituents present in *C. sinensis* L. dried extract was used (Patil *et al.*, 2013; Aslam & Afridi, 2017).

**Fluorescence analysis:** Powder study and fluorescence analysis were carried out with the help of different chemical reagents, as performed in protocol (Abbas *et al.*, 2015) of all four plant parts of *C. sinensis* L.

**Histological and microscopic analysis:** Fresh root, stem, leaves and seed were fixed for one day in formalin, acetic acid and 70% alcohol with composition (5:5:90). Transverse section of root, stem and leaf were made with the help of blade and razor method. Sections were stained with safranin and fast green dye (Sylvester & Ruzin, 1994) prior to successive elimination of contents present in each part of the plant. For microscopic observation chloral hydrate 75% solution was mixed with powdered drug. Slides of the powdered plant materials were prepared following the procedure of Ishtiaq *et al.*, (2018b).

**Thin layer chromatography:** For thin layer chromatography (TLC silica gel 60 F254 Merck Germany) different chemicals were purchased from local market. Different compounds which appeared on TLC plates under UV and Iodine chamber on TLC chromatogram were recorded according to the protocol (Abbas *et al*., 2015).

## Results and Discussion

**Macroscopic analysis:** *C. sinensis* L.is a woody plant attaining a height ca 15 m and 0.6-1.5 m in width (Mukhopadhyay *et al*., 2016). Leaves are simple, light green, alternate, short stalked, stipulate, coriaceous, texture leathery, glabrous at the upper surface, glabrous to glabrescent on the lower side oblong or elliptic with serrulate margins 5-9 cm long and 2-3.5 cm broad with cuneate base and acute to obtuse apex (Table 1).

Flowers white, fragrant, solitary axillary or in the form of clusters of two or four bisexual, about 2.5-3.5 cm in diameter, 0.6-1cm long. Fruiting starts after 5-6 years old plant. Fruits green in colour, smooth, flattened having 2-3 solitary seeds (Mondal *et al*., 2004). Ovary pubescent, three loculed with one orbicular seed in each locule (Ross, 2005; Biswas, 2006).

**Phytochemical investigations:** Phytochemical analysis of botanicals can play important role in the discovery of new therapeutic agents and also to explore the vital resources of economic materials. As the natural medicine has gained the fame of profitable busniness, their safety, quality, efficacy and therapeutic effectiveness have now become the matter of great concern. In the present study preliminary phytochemical study was performed on root, stem, leaf and seeds which showed the presence of flavonoids, alkaloids, carbohydrates,terpenoids, fats, fixed oils,proteins and steroids present in the plant (Table 2) which can be useful not only for treating different diseases but can be helpful as valuable herb for human consumption.

**Table 1. Morphological traits of different parts of *C. sinensis* L.**

| Plant part | Parameters | Results |
|---|---|---|
| Root  | Texture | Hard, scaly |
| | Color | Outer colour brown inner colour yellow whitish |
| | Taste | Bitter |
| | Odor | Pleasant? |
| Stem  | Texture | scaly, hard |
| | Color | Outer colour greyish brown, dark brown inner colour off white |
| | Taste | Astringent |
| | Odor | Pleasant? |
| Leaf  | Texture | Wrinkled, twist and furrows |
| | Color | Greenish |
| | Taste | Astringent/bitter |
| | Odor | Pleasant and fragrant smell |
| Seed  | Texture | Smooth |
| | Color | Outer color dark brown to black inner color reddish brown |
| | Taste | Astringent |
| | Odor | Dust like |

**Fluorescence analysis:** The fluorescent analysis can be used for the characterization of crude drug since it helps to identify different constituents in the plant material. Few chemical constituents showed fluorescence in daylight while some produced fluorescence in ultra violet light. Other chemical compounds were made fluorescent by the addition of different reagents (Table 3).

**Histological and Microscopic analysis:** The microscopic evaluation of all parts of *C. sinensis* L. was performed. Cell shape and size, and powder microscopy was conducted to determine the diagnostic features. The transverse section of root showed the major part was surrounded by vessels that were diffused in the central part. The large number of vessels in the root also showed its potential for the water absorption. The presence of vessels in diffused manner also indicated the secondary growth in root part. The transverse section of stem showed the circular vascular bundles, which indicated the presence of protoxylem and metaxylem. The presence of fibres in stem showed tannins. The transverse section of leaf indicated the isobilateral arrangement of epidermis. The vascular bundle of leaf showed metaxylem and protoxylem below; while phloem was present on the top in the leaf section. The root powder was pale yellow in colour, with a pleasant odour while bitter and astringent taste. The powdered study of root showed different structures, the diagnostic characters were cork cells, tracheid, periderm and vessels. The stem powder was green in colour, possessing pleasant odour and bitter taste. The leaf powder showed different structures, like tracheid, cortical cells, reticulate wall thickening vessels, spiral wall thickening vessels, annular wall thickening vessels and periderm tissue. The leaf powder was dark green in colour, having pleasant odour with bitter and astringent taste and showed different structures, like glandular trichomes and epidermal cells. The seed powder was off-white and brown in colour, having pleasant odour and bitter taste. The powdered study of seed showed abundant starch granules (Fig. 1).

**Table 2. Preliminary phytochemical investigation of *C. sinensis* L.root, stem, leaf and seed.**

| Phyto-constituents | Tests | Root | Stem | Leaf | Seed |
|---|---|---|---|---|---|
| Alkaloids | Mayer's test | ++ | + | - | - |
| | Wagner's test | ++ | ++ | - | - |
| | Hager's test | ++ | - | + | + |
| Glycosides | Fehling's test | +++ | - | ++ | - |
| | Keller killinani test | - | ++ | ++ | - |
| | Froth formation test | ++ | - | ++ | - |
| | Foam test | ++ | - | ++ | - |
| Tannins | Ferric chloride test | +++ | ++ | + | +++ |
| | Gelatin test | ++ | +++ | - | ++ |
| | Vanillin-HCL test | - | - | - | - |
| Resins | Acetone water test | ++ | - | ++ | ++ |
| Flavonoids | Lead acetate test | ++ | ++ | ++ | - |
| | Sodium hydroxide test | + | ++ | ++ | ++ |
| Lignin | Saffranine test | - | - | - | - |
| Protein | Xanthoproteic test | + | + | + | - |
| | Ninhydrin test | - | - | ++ | - |
| | Biurit test | - | - | ++ | - |
| Carbohydrates | Benedict's test | ++ | ++ | + | - |
| | Molisch's test | + | + | + | + |
| | Fehling's test | +++ | - | - | - |
| Tri-terenoid | Salkowskites | ++ | - | + | - |
| Steroid | Vanillin-$H_2SO_4$ test | - | - | - | - |
| Fats and fixed oils | Stain tests | - | - | - | |

The stem showed complete secondary growth with distinct medullary rays having vascular tissues in between the medullary rays. Pith was absent, few layers of periderm were observed around the vascular bundles. Periderm was composed of Phellogen, Phellem, and Phelloderm (Fig. 2A and 2C). Transverse section of root was almost elliptical in shape and showed marginal epidermal cells, inner cortex, pericycle and rounded pith cells (Fig. 2, D-F) Cross sectional view of leaf showed vascular bundles with xylem and phloem tissue in the central area, Xylem showed protoxylem and metaxylem surrounded by spongy rounded parenchyma with a single row of epidermal cells (Fig. 2, G-I). Trichomes were also observed on the surface of epidermal cells. Abundant stomata were seen in the lower epidermis (Fig. 2E).

**Thin layer chromatography:** Thin layer chromatography (TLC) is an easy, frequent and consistent method of analysis.TLC can be used for natural product extracts, finished product, stability testing, and stability testing of the extracts. On the basis of the retention factor value and colour spots TLC can play a vital role in the identification of components present in the plant. In the present study TLC finger prints of methanolic extracts of root, stem, leaf and seed of *C. sinensis* L. demonstrated the presence of complex mixtures of polar and non polar compounds in different solvent systems (Tables 4).

The compounds that appear in common and different bands are useful for the authentication of plant drugs (Tables 4, 5, 6 and 7). $R_f$ values (migration distance of individual compound) have been measured in the identifications the colour of the spot specific with a standard when silica plates were sprayed chromogenic reagents. The best identification depends on the positioning of the spot and the appropriate standard. The acceptance of the TLC procedure was adequate to detect the specific marker, with respect to the relative position of the band and the colour.

For the identification and authentication of pure drug plants, it is necessary to analyse the plant pharmacognosticaly. Such analysis helps to establish the quality standards for drug plants (Ishtiaq *et al*., 2018b). Microscopic and macroscopic evaluations provide the detailed information that delimits the very closely related species in the same genus (Shaheen *et al.*, 2017; Ishtiaq *et al*., 2018b). Anatomical studies i.e. powder microscopy and transverse sections of various parts of (root, stem, leaves and seed) of *C. sinensis* L were performed to set standards for its authentication. The root showed the presence of periderm containing phelogen, phellem and pheloderm (Fig. 2A and 2B) which is a protective tissue that replaces the epidermal layer when the cork cambium displaces the epidermal position in primary plant body. It secures the plant's internal body from the deleterious effects of the harsh conditions of the environment during the formation of the secondary plant body. Likewise, the collenchyma cells were also observed in the stem and leaf. For the mechanical support collenchyma tissues are present (Nikam *et al*., 2012). These tissues play an important role in the plants lacking secondary growth (Hanif *et al*., 2016).

The powder microscopy of leaf, stem and root enhance the structures of a drug when it is in macerated form, common structures viz., fibres, cork cells, vessels, parenchymatous tissues etc. (Fig. 1). Phellem commonly known as cork cells act as a protective sheath, fibres provides elasticity and support, parenchyma act as a storage tissue as well as photosynthesizing tissues, while tracheids with bordered pits and vessels are involved in the transportation of minerals (Mauseth, 1988; Sperry *et al*., 2006). Starch granules were also observed in microscopy. This depicts the nutritional value of the *C.*

*sinensis* L in terms of starch contents (Deatherage *et al*., 1955). To find out the fluorescent compounds fluorescence analysis with a mixture of chemicals was employed (Table 2) which confirmed their presence. The preliminary phytochemical screening of MeOH extract of whole plant exhibited the occurrence of carbohydrates tannins, flavonoids and sterols as major groups (Table 3). These phytochemicals are considered to be responsible for the various therapeutic effects that are ascribed to this plant material. TLC is a very popular method to check its wide variety of applications (Barbosa *et al*., 2011; Rubab *et al*., 2021). It is being used worldwide to check the quality of medicinal plants and herbal drug plants (Braz *et al*., 2012; Bahadur *et al.*, 2020).

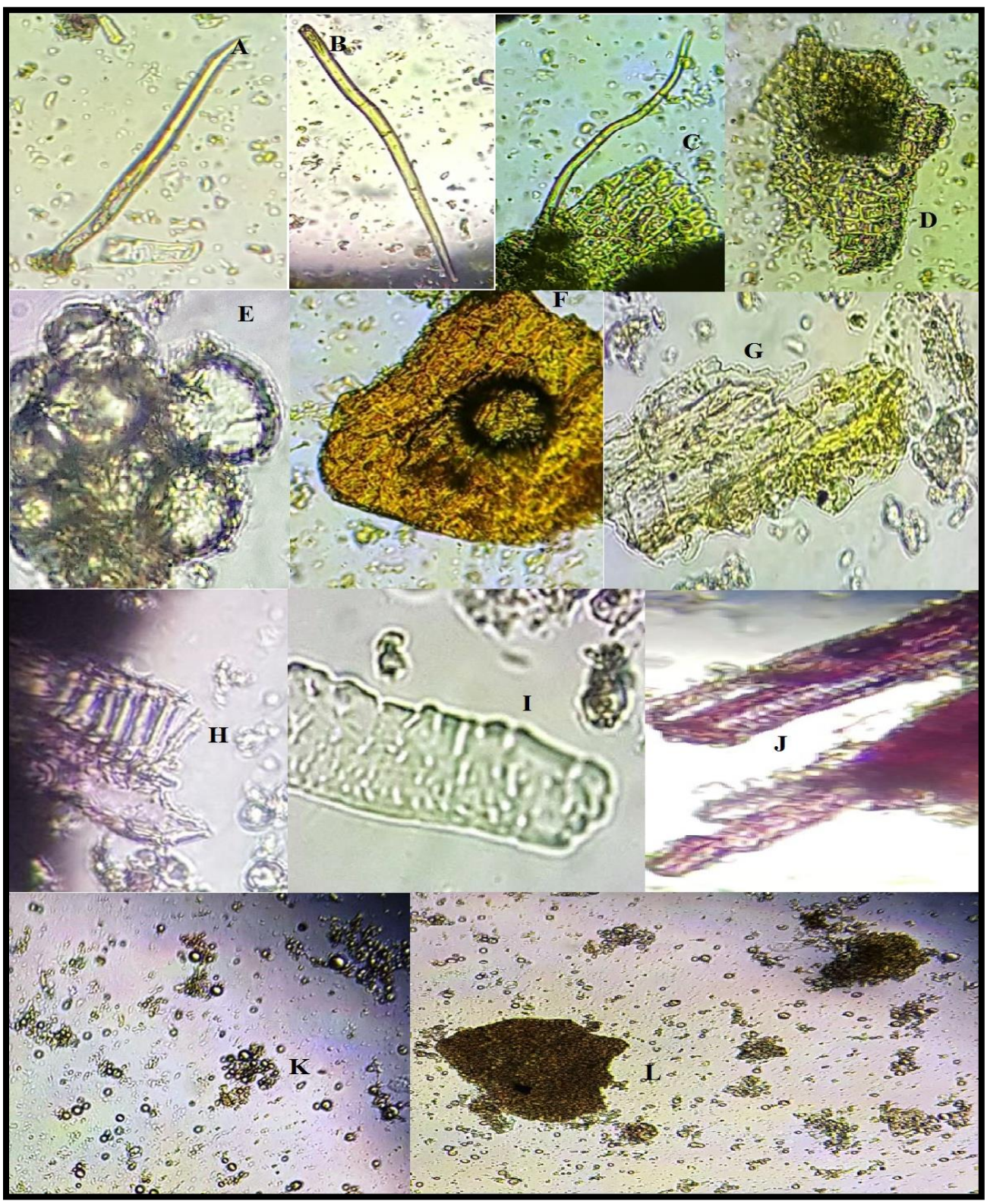


Fig. 1. Powder study of root, stem, leaf and seed. Here A and B are trichomes C: epidermal cells and trichome, D: epidermal cells E: cortical cells, F: priderm tissues, G: cork cells, H and I: spiral wall thickening vessels, J: annular wall thickening vessels, K and L: starch granules.

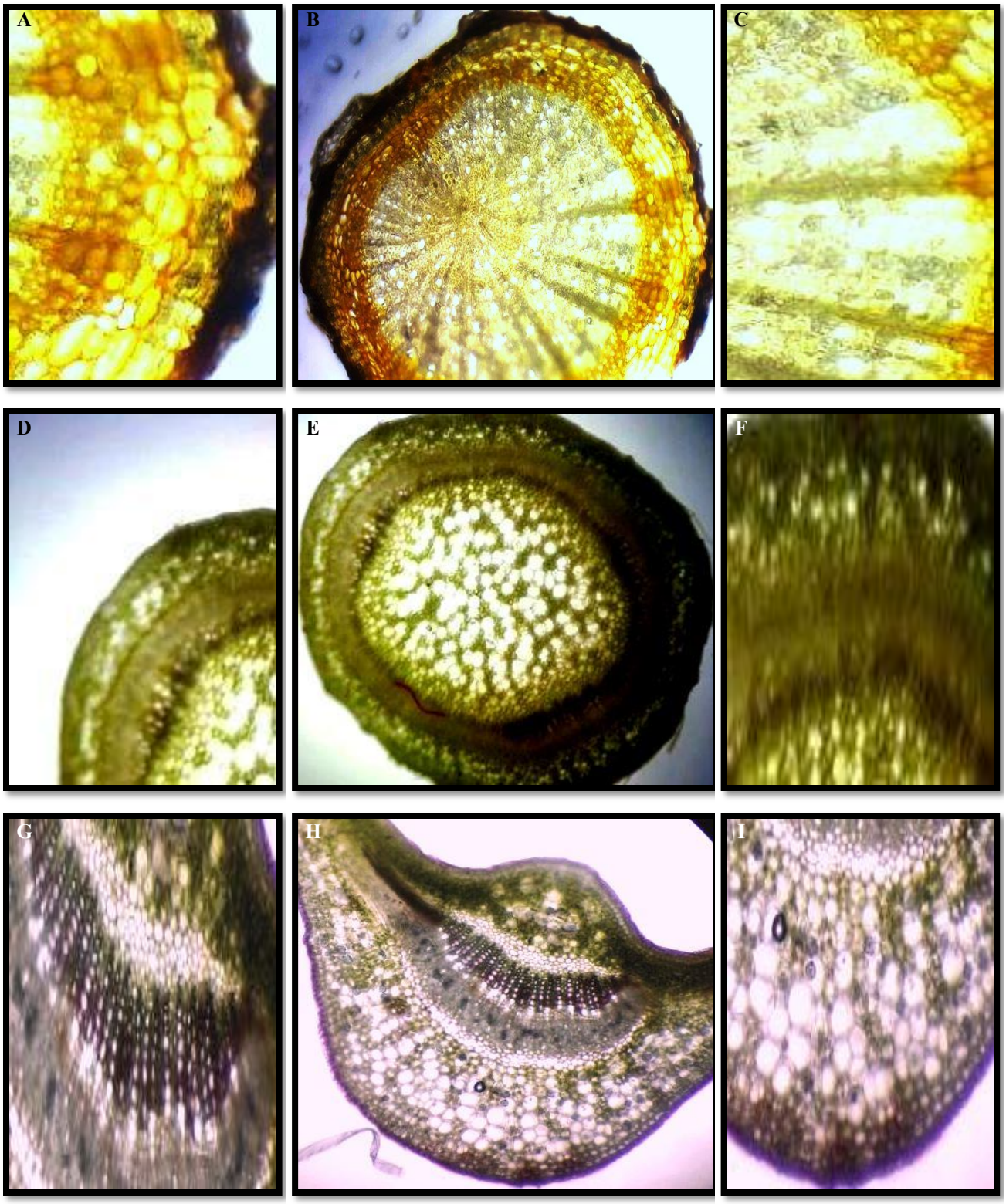


Fig. 2. Transverse sections of root (A-C), stem (D-F) and leaf (G-I) of *C. sinensis* L.

## Conclusion

Although tea had a great history of utilization a detailed investigations was attempted on the phytochemical parameters, macroscopic, microscopic features of root, stem, leaf and seed of *C. sinensis* L. for the first time in Pakistan. Morphological studies play an important role for the identification of the crude drug, while macroscopic features might be helpful for its implication against various diseases. The preliminary studies provide a valuable source of information not only to find the originality of the crude drug but also for determining the standards of the drug according to WHO guidelines. Present study can also be helpful for establishing the correlation between the photochemistry, phytotherapy, and pharmacology aspect of this amazing herb for the future research endeavours of the studied plant.

**Table 3. Fluorescence study of powdered *C. sinensis* L. root, stem, leaf and seed.**

| Sr. No. | Powder study Powdered drug + reagent | Root | | Stem | | Leaves | | Seed | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ordinary light | UV light | Ordinary light | UV light | Ordinary light | UV light | Ordinary light | UV light |
| 1. | Simple Powder | Light brown | Light brown | Brownish yellow | Brownish yellow | Dark green | Brown | Brownish yellow | Whitish brown |
| 2. | Powder + 1 N NAOH solution in water | Brown | Dark green | Yellowish brown | Dark green | Reddish brown | Black | Pale orange | Light green |
| 3. | Powder + 1N NAOH solution in methanol | Brown | Light yellow | Brown | Light yellow | Yellow green | Black | Yellow | White |
| 4. | Powder + 50% $H_2SO_4$ | Brown | Green | Brown | Green | Green | Green | Brownish yellow | Brownish yellow |
| 5. | Powder + 1N HCL | Brown | Green | Brown | Green | Green | Green | Brownish yellow | Brownish yellow |
| 6. | Powder + 50% $HNO_3$ Nitric acid | Yellowish brown | Blackish brown | Yellowish brown | Reddish brown | Brick red | Dark red | Brownish yellow | Reddish black |

**Table 4. Comparative thin layer chromatographic analysis of methanolic extract of *Camellia sinensis* L. root with different solvent systems.**

| Solvent system/Ratio | No. of comp. | Detection | | | Rf value |
|---|---|---|---|---|---|
| | | Naked eye | UV light | Iodine chamber | |
| BtOH: A.A: H2O 4:1:5 | 3 | Brown (1) | White (1) | Brown (1) | 0.2, 0.53, 0.53 |
| Hexane: E.A 9:1 | 7 | Light yellow (1) | Red, White, Red, Red (4) | Brown, Yellow (2) | 0.29, 0.02, 0.25, 0.66, 0.72, 0.33, 0.91 |
| E.A :MeOH: H20 8:1:0.1 | 6 | Yellow (1) | Purple, Pink (2) | Brown, Yellow, Dark brown (3) | 0.74, 0.77, 84, 0.03, 0.74, 0.81 |
| $CHCl_3$/MeOH 9:1 | 3 | - (0) | - (0) | Brown, yellow, Dark brown (3) | 0.03, 0.46, 0.86 |
| $CHCl_3$/MeOH 8:2 | 6 | Yellow (1) | Purple, Red (2) | Brown, Dark brown, Yellow (3) | 0.66, 0.66, 0.73, 0.06, 0.7, 0.76 |
| $CHCl_3$/MeOH 7:3 | 8 | Purple (1) | Purple, Pink (1) | Brown, Dark brown, Yellow, Dark yellow, Brown (6) | 0.82, 0.75, 0.82, 0.03, 0.17, 0.27, 0.44, 0.82 |

**Table 5. Comparative thin layer chromatographic analysis of methanolic extract of *C. sinensis* L.stem with different solvent systems.**

| Solvent system/Ratio | No. of comp. | Detection | | | Rf value |
|---|---|---|---|---|---|
| | | Naked eye | UV light | Iodine Chamber | |
| BtOH/EtOH/ H2O 9:1 :0.1 | 4 | Green (1) | Pink (1) | Yellow, Brown (2) | 0.67, 0.71, 0.13, 0.63 |
| BtOH: A.A: H2O 4:1:5 | 3 | Orange-Yellow (1) | Light Purple(1) | Yellow (1) | 0.64, 0.57, 0.57 |
| Hexane: E.A 9:1 | 13 | Light yellow, Pale yellow, Dark green (3) | Purple, Dark red, Light red, Red, Light purple (5) | Brown, Yellow, Dark brown, Yellow, Light brown (5) | 0.3, 0.27, 0.41, 0.03, 0.16, 0.27, 0.41, 0.48, 0.24, 27, 0.21,0. 62, 0.87 |
| E.A: MeOH: H20 8:1:0.1 | 5 | Pale yellow, Yellow (2) | Purple, Pink (2) | Dark yellow, Yellow (2) | 0.01, 0.80, 0.78, 0.07, 0.80 |
| $ChCl_3$/MeOH 9:1 | 5 | Light green (1) | Dark pink (1) | Brown, Yellow, Dark brown (3) | 0.74, 0.79, 0.03, 0.62, 0.75 |
| $CHCl_3$/MeOH 8:2 | 10 | Purple, Yellow, Green (3) | Purple, Pink, Light pink, Red (4) | Brown, Dark Yellow, Dark brown (3) | 0.62, 0.66, 0.73, 0.16, 0.56, 0.63, 0.76, 0.03, 0.53, 0.73 |
| $CHCl_3$/MeOH 7:3 | 9 | Green, Light yellow (2) | Purple, Pink (2) | Brown, Dark brown, Yellow, Dark yellow, Yellow (5) | 0.17, 0.96, 0.75, 0.82, 0.02, 0.08, 0.20, 0.75,0. 89 |

**Table 6. Comparative thin layer chromatographic analysis of methanolic extract of *C. sinensis* L. leaf with different solvent systems.**

| Solvent system/Ratio | No. of comp. | Detection | | | Rf value |
|---|---|---|---|---|---|
| | | Naked eye | UV light | Iodine Chamber | |
| BtOH/EtOH/ H2O 9:1 :0.1 | 7 | Green, Yellow, Pink (3) | Dark Red (1) | Dark brown, Brown, Yellow(3) | 0.32, 0.46, 0.57, 0.57, 0.39, 0.53, 0.57 |
| BtOH:A.A: H2O 4:1:5 | 3 | Bright yellow (1) | Brown (1) | Dark brown (1) | 0.71, 0.53, 0.53 |
| Hexane: E.A 9:1 | 11 | Bright yellow, Yellow-green, Dark green (3) | Red, Dark Red, Pink, Pink, Dark pink (5) | Brown, Yellow, Dark yellow (3) | 0.12, 0.31, 0.45, 0.01, 0.13, 0.18, 0.31, 0.44, 0.10, 0.27,0. 45 |
| E.A:MeOH: H20 8:1:0.1 | 12 | Yellow, Yellow, Green, Yellowish-green(4) | Dark red, Dark red, Reddish-brown, Pink (4) | Brown, Dark brown, Yellow, Dark yellow (4) | 0.14, 0.45, 0.49, 0.77, 0.14, 0.46, 0.56, 0.77, 0.07, 0.21, 0.42,0. 66 |
| $CHCl_3$/MeOH 9:1 | 11 | Dark green, Yellow, Green (4) | Light pink, Slightly Light pink, Dark pink (3) | Brown, Dark brown, Light yellow, Yellow brown (4) | 0.55, 0.68, 0.77, 0.11, 0.55, 0.79, 0.13, 0.41, 0.51, 0.61, 0.77 |
| $CHCl_3$/MeOH 8:2 | 14 | Light brown, Light yellow, Green, Bright yellow, Dark green (5) | Dark red, Red, Dark red, Red (4) | Brown, Dark brown, Pale brown, Yellow, Light yellow (5) | 0.13, 0.26, 0.50, 0.66, 0.73, 0.16, 0.56, 0.63, 0.76, 0.13, 0.23, 0.50, 0.53, 0.73 |
| $CHCl_3$/MeOH 7:3 | 12 | Green, Yellow, Green, Purple (4) | Dark brown, Dark red, Pink, Light Pink(4) | Dark brown, Yellow, Brown, Dark yellow(4) | 0.10, 0.15, 0.31, 0.75, 0.24, 0.68, 0.75, 0.84, 0.55, 0.65, 0.68, 0.89 |

**Table 7. Comparative thin layer chromatographic analysis of methanolic extract of *C. sinensis* L. seed with different solvent systems.**

| Solvent system/Ratio | No. of comp. | Detection | | | Rfvalue |
|---|---|---|---|---|---|
| | | Naked eye | UV light | Iodine Chamber | |
| BtOH/EtOH/ H2O 9:1 :0.1 | 5 | Pink (1) | White (1) | Yellow, Yellow, Brown (3) | 0.67, 0.53, 0.23, 0.60, 0.62 |
| BtOH: A.A: H2O 4:1:5 | 3 | Pink (1) | White (1) | Brown (1) | 0.66, 0.53, 0.53 |
| Hexane: E.A 9:1 | 2 | - (0) | White (1) | Brown (1) | 0.02,0. 91 |
| E.A:MeOH: H20 8:1:0.1 | 9 | Purple (1) | White, White, White(3) | Brown, Light brown, Yellow, Pale yellow (4) | 0.25, 0.03, 0.10, 0.13, 0.03, 0.13, 0.23, 0.33, 0.91 |
| $CHCl_3$/MeOH 9:1 | 3 | Purple (1) | - (0) | Brown, Brown, Yellow (2) | 0.08, 0.13, 0.86 |
| $CHCl_3$/MeOH 8:2 | 3 | - (0) | White (1) | Brown, Yellow (2) | 0.08, 0.06, 0.75 |
| $CHCl_3$/MeOH 7:3 | 6 | Purple (1) | White (1) | Brown, Yellow, Dark brown, Dark yellow (4) | 0.82, 0.17, 0.02, 0.06, 0.34, 0.82 |

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