Phytochemical and Pharmacological Potential of Camellia sinensis L.

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ABSTRACT

Crude solvent extracts of traditional medicinal plants have been used for thousands of years in different regions of the globe for the treatment of various diseases. In developing countries, traditional medicines are used as source of primary health care. Keeping in view the importance of Camellia sinensis L., present investigation was aimed to evaluate the phytochemical and pharmacological potential of different morphological parts of C. sinensis L. Successive extractions of all plant parts was performed with different solvents like, petroleum ether, acetone, ethanol and water. Phytochemical analysis of all extracts showed the presence of polyphenolic compounds, flavonoids, alkaloids, glycosides and carbohydrates in all plant parts with varied strength. Phytochemical analysis showed comparatively high percentage yield of ethanolic extract of all plant parts, hence was employed for evaluating the antimicrobial potential against 11 Gram positive bacteria, 9 Gram negative bacteria, 2 yeasts, 2 dermatophytes and 7 saprophytes. The petroleum ether seed extract and methanolic leaf extract was evaluated for the comparative anti-inflammatory and analgesic potential using different parameters like licking - biting response, inflammation of hind paw and writhing effect. The results showed that different pharmacological activities were due to the presences of various phytochemicals like tannins, resin and flavonoids, observed maximally in ethanolic extract with minor quantity of alkaloids and glycosides. Fourier transform infrared spectroscopy (FTIR) of dried roots, stems, leaves and seeds provided a conclusive support to the above results. Anti-inflammatory effect was less significant (p < 0.05) in seed extract, while leaf extract displayed highly significant results both at low and high doses. Likewise, both seed and leaf showed significant analgesic effects. However, compared to seed extract which showed highly significant (p < 0.001) increase in concentration dependent manner, leaf extract displayed highly significant results even at low dose with better results at high dose compared with standard.

INTRODUCTION

Improper and extensive use of antimicrobial agents has led to the emergence of unusual infections with strong adverse effects. Plants can be considered as a good option as antimicrobial agents (Olila and Opuda-Asibo, 2001; Pawar and Nabar, 2010). Elevated expenditure and side effects associated with synthetic medicines are attracting the researchers to search for alternative antimicrobial drugs (Ponnusamy et al., 2010; Janakiraman et al., 2012).

The proper functioning of human body requires the essential components which play vital role in the growth and development. The consumption of herbal medicine has gained particular attention in medical practice due to its growing knowledge and universal tolerability (Folashade et al., 2012; Liaqat et al., 2017; Iqbal et al., 2020). The natural constituents are relatively more compatible and healthier as compared with synthetic one (Liaqat et al., 2017; Ijaz et al., 2021). Secondary metabolites such as alkaloids, polyphenols tannins and flavonoids obtained from the herbal medicinal plants provide the important role in the evaluation of biological and pharmacological activities (Liaqat et al., 2017; Rubab et al., 2017). There is
need for the identification of the natural products obtained from the herbal medicinal plants which have significant role in treating the pain and inflammatory disorders and have non-addictive properties with lesser side effects (Chen et al., 2015; Paliwal et al., 2017). Extensive work is being done for the identification of important phytochemical, antimicrobial and antifungal components from diversities of normal flora found around the world.

Colourful fruits, vegetables, spices, wines and teas contain dietary polyphenols, which have gained the fame due to their powerful anti-inflammatory and antioxidant activity (Wollen, 2010). These natural products can be utilized as therapeutic agents and replica for the compounds which are pharmacologically active and can be utilized for the synthesis of the synthetic drugs (Kerwat et al., 2010).

Pharmacognostic methods are helpful in minimizing the phytochemical variations. Phytochemical analyses are helpful in evaluating the percentage and efficacy of biomolecules present in various parts of the plant. Camellia sinensis L. (green tea) is cultivated in Shinkiari, District Mansehra, Pakistan (Rubab et al., 2020a). It is the rich source of polyphenols, flavonoids, polysaccharides and anthraquinone derivatives. Its leaves are used as beverage and the catechins are an important component (Xiong et al., 2013). Components of this plant predominantly include polyphenols, caffeine and amino acids, which are beneficial for human health (Tatiya et al., 2017).

Therefore, present investigation was aimed to elaborate the phytoconstituents of Camellia sinensis L. (green tea) and to explore their potential role towards human benefits. The variations present in the phytochemical and antimicrobial potential of different morphological parts of the green tea from the region, especially Pakistan were explored which to our knowledge have not been reported before. However, the analgesic and anti-inflammatory effects of its seed extracts have been reported occasionally.

**MATERIALS AND METHODS**

**Preparation of plant extract**

Camellia sinensis L. was collected from National Tea and High Value Crops Research Institute (NTHRI) Shinkiari, District Mansehra, Pakistan. All plant parts were kept in shade for about 15 days, dried completely and ground separately to a fine powder by passing through sieve no. 120. The fine-powdered plant materials were stored in amber coloured bottles and well-preserved at ambient temperature and pressure.

All powdered plant parts (500g) like root, stem, leaf and seed were subjected to extraction at room temperature using 3 L of each solvent like petroleum ether, acetone, ethanol and water, based on their polarity the successive extractions were carried out. The extraction was done with Soxhlet apparatus at 40°C with 40 rpm and at 0.9 MPA pressure. All powdered materials were macerated for four days at room temperature and were filtered. The filtrate was dried at reduced pressure and residues were obtained which were weighed and stored in the air tight containers at room temperature.

Phytochemical analysis was performed for the determination of the presence of primary and secondary metabolites like, carbohydrates, fats, fixed oils, proteins flavonoids, glycosides, resins, alkaloids, terpenoids and steroids present in plant parts.

**Screening of antimicrobial activity**

Agar well diffusion method was used for the screening of the antimicrobial activity of Camellia sinensis L. against a library of microbial cultures, preserved at the Department of Microbiology, University of Karachi, Pakistan. The antimicrobial activity of the extracts was tested against 11 Gram positive bacteria (Bacillus subtilis, B. cereus, Staphylococcus aureus, S. saprophyticus, S. epidermidis, Enterococcus faecalis, Vancomycin Resistant Enterobacter (VRE), Meticillin Resistant S. aureus (MRSA), Micrococcus luteus, Corynebacterium xerosis), 9 Gram negative bacteria (Salmonella typhi, S. paratyphi A, S. paratyphi B, Shigella sp., Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Enterobacter sp, Pseudomonas aeruginosa), two yeasts (Candida albicans and Saccharomyces cerevisae), two dermatophytes (Trichophyton mentagrophyte and Microsporum gypseum) and seven saprophytes (Aspergillus niger, Penicillium sp., Puccilomyces varioli, A. flavus, Chrysosporium sp., Fusarium oxysporum, Chrysosporium sp., A. terreus, A. tereblica).

Stock solution (20 mg/mL) of plant extracts was prepared in sterilized 40% dimethyl sulfoxide (DMSO, Sigma Aldrich, UK). DMSO was used as a negative control, while ciprofloxacin (CIP, Oxoid, UK) and Otosporin (Oxoid, UK) was used as a positive control for bacteria and fungi, respectively.

The cell suspension was prepared by inoculating 24 h old bacterial culture and yeast (0.5 McFarland standards) in 5 mL saline. For fungal cultures, spore suspension (5 x 10⁶ spores/mL) was prepared from 5 days old mould plates. Confluent lawn was made on Mueller Hinton Agar (MHA, Oxoid, UK) plates and Sabourauds Dextrose Agar (SDA, Oxoid, UK) for bacterial and fungal cultures, respectively, and permitted to dry for 5-10 mins. Wells (8 mm) were made with the help of sterile borer. Plant extracts, DMSO solution, positive or negative controls (20 µL) were added into the wells. Plates were incubated at 37 °C for 24 h for...
bacteria and 5 days for fungi (Liaqat et al., 2017).

**Determination of minimum inhibitory concentration (MIC)**

MIC was determined via agar well diffusion assay. The extracts exhibited a significant zone of inhibition (>15 mm). The extracts were dissolved in sterile DMSO with 20 mg/mL concentration and serially diluted to 1.25 mg/mL. The above-mentioned method was repeated to observe the maximum dilution that had not displayed slightly noticeable turbidity, which was considered as MIC.

**Determination of minimum microbicidal concentration (MMC)**

In order to understand whether the appearance of the zone of inhibition is due to the death of organisms or timely halt of microbial growth, MMC was determined. A loop streak from the zone of inhibition was inoculated on fresh appropriate media. After 24 h, observed growth suggested microstatic activity of plant extracts while absence of growth showed lack of microbicidal activity.

**Fourier tranform infrared spectroscopy (FTIR)**

FTIR (Agilent, Cary 360) was used for the identification of functional groups and their positions in the Phyto molecule. The operating range of FTIR was between 4000-650 cm⁻¹ (Liaqat, 2009). Fine powder of root, stem, leaf and seed was used for the FTIR spectroscopic analysis.

**Animals (in vivo study)**

Seventy two male albino mice weighing 20-22 g were used as test animals in the current study for anti-inflammatory and analgesic activities. They were bought from Dow University, Karachi and distributed into 2x six groups (6 mice per group). The 2x six groups were categorized as control, seed at low dose, seed at high dose, leaf at low dose and leaf at high dose for each of anti-inflammatory and analgesic activity, respectively. The standard laboratory settings such as 25°C temperature; light and dark cycles of 12 h were maintained and food and water was provided *ad libitum*. Mice were acclimatized for 15 days prior to study. Ethical approval for present investigation was provided by University of Karachi via letter No. 1227/19.

**Determination of anti-inflammatory and analgesic potential of seed and leaf extract**

For the determination of anti-inflammatory and analgesic potential of *C. sinensis* L. ethanolic extract of seed and leaf were used. For anti-inflammatory activity diameter of right hind paw of mice was noted with the help of vernier calliper prior to the dose of 2% formalin was injected in the subplater of the right hindpaw of controlled mice for inducing pain. Thirty mins before injecting the formalin, 300 mg/kg, and 600 mg/kg of both seed and leaf extracts of *C. sinensis* L. were given orally to all mice groups except for control. Licking response along with time duration was noted in two phases, early phase 0-300 s and late phase 900-1800 s (Ganeshpurkar and Rai, 2013; Mustaffa et al., 2010).

**Acetic acid-induced writhing effect**

Acetic acid (0.6%) in distilled water was injected intraperitoneally in mice using aspirin (10 mg/kg) as standard. Acetic acid was calculated for each individual mouse according to body weight. Rest of the procedure is same as mentioned above except for that calculated dose of acetic acid was injected instead of formalin. Abdominal constriction in mice was noted in two phases, early phase 0-300 s and late phase 900-1800 s (Mumtaz et al., 2017).

**Statistical analysis**

The results of the antimicrobial activity of extract were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using IBM SPSS statistics version 20 (Rubab et al., 2020b). Significant differences between and within group were measured using one way analysis of variance (ANOVA) followed by Post hoc LSD for all groups at p < 0.05.

**RESULTS**

The ethanolic extracts of *C. sinensis* L. seeds showed high percentage yield compared to other solvents. The lowest percentage yield was obtained by petroleum ether extract fractions (Supplementary Table I). Phytochemical analysis of petroleum ether extracts of the parts of the plant showed positive results for primary metabolites while secondary metabolites indicated lack of lignin and steroids (Supplementary Table II). The acetone extract of the parts of the plant showed negative result for protein and provided positive results for secondary metabolites (Supplementary Table III). The ethanolic extracts of the parts of the plant indicated negative results for fat and fixed oil while among secondary metabolites, lignin proteins, resins and steroids were absent (Supplementary Table IV). The water extracts of the parts of the plant showed negative results for primary metabolites like, fat and fixed oil while secondary metabolites showed lack of steroids (Supplementary Table V).

**Antimicrobial activity**

The antimicrobial activity of the ethanolic extracts of root, stem, leaves and seeds of *C. sinensis* L. was assessed by agar well diffusion technique. Library of test organisms comprised of 10 Gram positive, 09 Gram negative bacteria,
2 dermatophytes, 2 yeasts and 8 opportunistic pathogenic moulds.

The ethanolic extract of root and stem of *C. sinensis* has shown strong activity against *C. xerosis* while moderate for *B. cereus*. Similarly, the ethanolic extract of leaves has revealed a bigger zone of inhibition (ZOI) for *S. typhi* (20.6±1.0) and *Para typhi A* (25±0) while moderate against *E. coli* (14.6±0.5) and *K. pneumoniae* (15.0±1). No activity was shown by the ethanolic extract of seeds. It can be inferred from the results in (Table I) the ethanolic extracts of different parts of the *C. sinensis* L. has not shown significant results against most of the bacterial cultures except for the few as mentioned above.

Table I. Antibacterial activity of ethanolic extracts of various parts of *C. sinensis* L against Gram positive and Gram negative bacteria as demonstrated by zones of inhibition (ZOI) in mm.

| Bacteria          | ZOI by plant extracts (mm) |
|-------------------|----------------------------|
|                   | Seed | Root | Stem | Leave |
| **Gram positive bacteria** |       |       |       |       |
| *B. cereus*       | - 13.6±1.15  | 14.2±0.6 | -     |       |
| *B. subtilis*     | -    | -    | -    | -     |
| *S. aureus*       | -    | -    | -    | -     |
| *S. epidermidis*  | -    | -    | -    | -     |
| *S. saprophyticus*| -    | -    | -    | -     |
| MRSA              | -    | -    | -    | -     |
| *M. leteus*       | -    | -    | -    | -     |
| VRE               | -    | -    | -    | -     |
| *E. faecalis*     | -    | -    | -    | -     |
| *C. xerosis*      | - 20.3±0.5  | 25±1.1 | -    | -     |
| **Gram negative bacteria** |       |       |       |       |
| *S. typhi*        | -    | -    | -    | 20.6±1.0 |
| *Shigella sp.*    | -    | -    | -    | -     |
| *Para typhi A*    | -    | -    | -    | 25±0  |
| *Para typhi B*    | -    | -    | -    | -     |
| *E. coli*         | -    | -    | -    | 14.6±0.5 |
| *P. aeroginosa*   | -    | -    | -    | -     |
| *P. mirabilis*    | -    | -    | -    | -     |
| *K. pneumoniae*   | -    | -    | -    | 15.0±1 |
| Enterobacter sp.  | -    | -    | -    | -     |

>10, no activity; < 10, Slightly active; <13, moderately active; ≤15, strongly active.

All plant part ethanolic extracts exhibited antifungal properties against *C. albicans* and *S. cerevisiae*. Stem extract of the plant showed significant activity against *C. albicans*, *S. cerevisiae*, *Penicillium* sp. *A. flavus*, *F. oxysporum* and *A. terricola*. While remaining microbial cultures showed less or no ZOI against the tested extracts (Table II).

Table II. Antifungal activity of ethanolic extracts of various parts of *C. sinensis* L against dermatophytes, yeasts and opportunistic pathogenic molds as demonstrated by zones of inhibition (ZOI) in mm.

| Fungi             | ZOI by plant extracts (mm) |
|-------------------|----------------------------|
|                   | Seed | Root | Stem | Leave |
| **Dermatophytes** |       |       |       |       |
| *T. mentagrophytes* | -   | -    | -    | -     |
| *M. gypseum*      | -    | -    | -    | -     |
| **Yeasts**        |       |       |       |       |
| *C. albicans*     | 15.2±0.5 | 18.6±0.5 | 11.4±0.3 | 10.3±0.3 |
| *S. cerevisiae*   | 10.3±0.6 | 21.5±1.5 | 12.0±1  | 16.0±1  |
| **Oppurtuni**     |       |       |       |       |
| *A. niger*        | -    | -    | -    | -     |
| *Penicillium sp.* | -    | 10.0±1 | -    |       |
| *B. variotii*     | -    | -    | -    | -     |
| *A. flavus*       | -    | 11.3±0.5 | -    | -     |
| *Chrysosporium sp.* | -  | -    | -    | -     |
| *E. oxysporum*    | -    | 13.6±1.5 | -    | -     |
| *A. terreus*      | -    | -    | -    | 11.3±0.5 |
| *A. terricola*    | -    | 10±0.6 | -    | -     |

>10, no activity; < 10, Slightly active; <13, moderately active; ≤15, strongly active.

**MIC and MMC**

The results of MIC suggested that extracts possess antimicrobial potential at higher concentrations including 20, 10 mg/mL and significantly low potential at 5 mg/mL. Most of the MMC were same as that of MIC except for root extract against *C. xerosis* and seed extract against *C. albicans*. *C. albicans* was sensitive to root extracts of the plant at a minimum concentration of 2.5 mg/mL (Table III).

**FTIR study of *C. sinensis* L.**

FTIR analysis of different powdered parts (root, stem, leaf and seed) of the plant of *C. sinensis* L. showed the presence of different functional groups (Fig. 1, Supplementary Table IV).

**Anti-inflammatory activity**

The effect of ethanolic extracts of seed and leaf of *C. sinensis* L. on licking and biting response following formalin injection along with time duration was noted in four mice groups in two phases categorized as (0-300s) early phase and late phase (900-1800s) at low (300 mg/kg) and high dose (600 mg/kg) except for control and standard
groups. Control group mice showed 55.17 ± 1.25 values for licking and biting for time duration of 123.83 ± 2.51 and 26 ± 0.89 for time duration of 46.83 ± 2.66 during the early and late phases, respectively. At 300 and 600 mg/kg of seed extracts, the number of licking was observed to be 78.5 ± 2.20 for time duration of 90.5 ± 2.23, and 56.17 ± 3.79 for time duration of 46 ± 0.26, respectively. Likewise, standard mice group showed licking and biting response of 62.0 ± 0.73 for time duration of 94.50 ± 0.22 during early phase (Fig. 2). All mice groups showed response in late phase (Data not shown). The group injected by leaf extract showed numbers of licking and biting 35.17 ± 1.25 for time duration of 35.33 ± 1.20 and 39.0 ± 0.37 for time duration of 45.17 ± 0.31 during early phase at 300 and 600 mg/kg, respectively. The seed extract showed significant effect during the first phase compared to control and standard, while the significant effect was seen in the case of leaf extract at p < 0.01 both at low and high dose (Fig. 2).

### Table III. Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of *C. sinensis* L. ethanolic extracts.

| Microorganisms         | Ethanolic extracts of plant parts | MIC (mg/ml) | MMC (mg/ml) | ZOI (mm) |
|------------------------|----------------------------------|-------------|-------------|---------|
| *C. xerosis*           | Root                             | 10          | 20          | 12      |
|                        | Stem                             | 40          | 40          | 20      |
| *E. coli*              | leaves                           | 5           | 5           | 15      |
| *S. typhi*             | leaves                           | 10          | 10          | 12      |
| *S. para typhi A*      | leaves                           | 5           | 5           | 16      |
| *K. pneumoniae*        | leaves                           | 5           | 5           | 14      |
| *C. albicans*          | Seed                             | 5           | 10          | 13      |
|                        | Root                             | 2.5         | 2.5         | 16      |
| *S. cerevisiae*        | Root                             | 5           | 5           | 18      |
|                        | Leaf                             | 5           | 5           | 12      |

**Effect of *C. sinensis* L seed and leaf extract on oedema of right hind paw of mice**

The results from anti-inflammatory activity revealed that pain persisted after injecting 0.2 mL of formalin injection in the subplater of the right hind paw of controlled mice group from 0 to 150 mins. However, no difference was observed in mean values of inflamed hind paw of mice (Fig. 3).

The results were significant in the case of low dose of 300 mg/kg, high dose of 600 mg/kg and standard dose of aspirin. There was reduction in the inflammation of hind paw compared with control from 0 min to 150 mins and there was significant difference in the mean values of inflamed hind paw of mice. Post Hoc LSD revealed that at low dose result was insignificant when multiple comparisons were made between 30 and 60, 90 and 120 mins, having almost same values of mean diameter of hind paw (Fig. 3).

**Fig. 2.** Effect of *C. sinensis* L. ethanolic seed and leaf extract on licking and biting response along with time duration in six mice groups in two phases categorized as (0-300s) early phase at low (300mg/kg) and high (600mg/kg) doses, respectively. Seed treated mice group at high dose showed significant decrease in licking and biting responses at high dose, while leaf group showed significant decrease at both low and high doses compared to control and standard groups.

The seed extract at low dose displayed the substantial effect, while highly significant effects were observed at high dose compared to control and standard. The leaf extract displayed better effect at low dose in comparison with the high dose (Fig. 3).

**Acetic acid induced writhing effect**

Following intraperitoneal injection of acetic acid in mice, the comparative number of writhing and time duration were noted in two phases; the early phase (0-300 s) and the late phase (900-1800 s) for seed and leaf.
At 300 and 600 mg/kg of seed extracts, the number of writhing and the time duration for early phase was observed. Mice showed writhing values of 71 ± 0.37 for the time duration of 11.50 ± 0.62 and 52.67 ± 0.95 for the duration of 4.67 ± 0.21, at aforementioned two doses, respectively. Similarly at 300 mg/kg the values for writhing were 7.33 ± 0.21 for the time duration of 33 ± 1.15 and at 600 mg/kg, the writhes were 2.50 ± 0.22 for the time duration of 13.33 ± 1.26 were noted for the leaf extracts at two doses during early phase. Mice stopped writhing at the later phase. The standard group had shown writhing values of 11.5 ± 0.62 for the time duration of 71 ± 0.3 in early phase with zero writhing in the later phase (Fig. 4).

Significant effect (p < 0.05) of seed extract both at low and high dose was observed in all groups compared to the control and the standard groups. Interestingly, the low dose of leaf extract exhibited highly promising (p < 0.001) effects even at low concentration. As there was considerable reduction in the number of writhes both in early and late phases of leaf extract (Fig. 4).

DISCUSSION

Camellia sinensis L. has great therapeutic significance due to its pharmacological activities. Intensive research for new and more effective agents to deal with the infectious diseases caused by multiple drug resistant microorganisms is underway and plants are being reported as novel source of potentially useful medicinal compounds (Liaqat et al., 2017). Hence it is extremely essential to make use of these natural resources to combat the threat caused by resistant microbes. Natural compounds are not only cost effective but also provide a safe means of healing without any side effects.

Phytochemical screening of the C. sinensis L. with different solvents systems displayed the occurrence of tannins, flavonoids, glycosides; carbohydrates and lignin in almost all parts of the plant in all solvent system used during successive extraction procedure (Zulqarnain et al., 2021). Ethanol was the best solvent for extraction because it gives the highest percentage yield, but at the same time, not suitable media for seed extraction as seed shows less antimicrobial effect with most of the strains. These findings are considered to be the responsible for different therapeutic effects that are attributed to this plant (Ishtiaq et al., 2018).

Camellia sinensis L. is known for its inhibition potential against various bacterial species and possess anti cariogenic activity. It is the second most consumed drink of the world which is categorized by the occurrence of numerous constituents having anti-aging, anti-Alzheimer, anti-Parkinson, anti-stroke and anticancer properties.
(Gupta and Kumar, 2017). The dried leaves have revealed direct bactericidal effect against tested pathogens in the current study. This verifies with Hamilton-Miller (2001) who has reported that tea plant inhibits Streptococcus mutans and S. sobrinus with teeth. The plant has displayed potential antimicrobial activity against MDR bacterial strains which is similar to (Faroqui et al., 2015) who had also observed antimicrobial potential by C. sinensis L. against various pathogens. Antimicrobial activities of C. sinensis have been reported against various pathogenic bacteria including MRSA and MDR P. aeruginosa (Radji et al., 2013). Our results suggested strong antimicrobial activity against C. xerosis, S. typhi, K. pneumoniae, E. coli, and S. para typhi A., and did not show ZOI against P. aeruginosa, which could be due to the variation in the extraction methodology or the concentration used (Liaqat et al., 2017).

C. sinensis L. comprises naturally active constituents that might be responsible for the better antioxidant and antibacterial properties (Mahmood et al., 2010). Previously, it was reported that its constituents are major contributors towards the polymeric tannins (PT) and have robust antioxidant and antibacterial activities (Chan et al., 2011). The present study has shown strong activity against yeasts but moderate for opportunistic moulds which is in accordance to the previous studies have suggested the antifungal components may be used in developing the novel fungicides (Saha et al., 2005).

The FTIR analysis of the dry powdered parts of C. sinensis L. revealed the presence of absorption frequencies of organic compounds with functional groups of alcohols, amides, carboxylic acids, alkenes, alkanes, ketones, esters, aromatic amines, aromatic alkanes and anhydrides etc., as confirmed from the literature (Yashin et al., 2015). It can also be observed that the functional groups of roots and stems are very much similar.

The prevalence of abundant adverse effects of the conventional use of analgesic and anti-inflammatory drugs led to the discovery of biomolecules with significant good effects and negligible adverse effects (Boussouf et al., 2017). The present study is based on the evaluation of polyphenols present in C. sinensis seed and leaf extracts using acetic acid induced writhing effect and oedema of hind paw of mice. Both seed and leaf extracts have shown the dose dependent effect on inflammation and analgesia. The present investigation revealed that both the seed and leaf extracts of C. sinensis had shown the anti-inflammatory and analgesic effects. The phytochemical analysis has shown that both the seed and leaf extracts are rich in polyphenols and flavonoids. Seed extracts has exhibited better results at high dose while leaf has displayed highly significant outcomes even at low dose compared to the controlled and the standard groups. So, seed extract has shown less anti-inflammatory effect and more analgesic effect as compared to leave extract. This is in agreement with the findings of (Xiong et al., 2013) who had reported the presence of polyphenols, flavonoids, polysaccharides and anthraquinone derivatives. Catechins conferring anti-inflammatory and analgesic effects.

The highest percentage inhibition of writhing effect following dose of 600 mg/kg was noted. A significant reduction in oedema was noted at 300 mg/kg and 600 mg/kg dose of both the seed and leaf extracts. The results have shown significant reduction in sign and symptoms of tested mice groups which suggested that polyphenols present in C. sinensis seed and leaf extracts possess anti-inflammatory and analgesic effects (Xiong et al., 2013). The innate immunity plays key role in producing the clinical symptoms like heat, pain, oedema and redness as inflammation is body defence mechanism which is produced in the body to isolate and repair the affected tissue damage. Abundant release of inflammatory mediators results in persistent inflammatory effect which produces the symptoms of chronic inflammation (Youngebare-ziebrou et al., 2016).

For evaluating the analgesic effect of seed and leaf extracts, acetic acid induced writhing effect was monitored. It was used as a model of visceral pain and found associated with very sensitive mechanism to evaluate the analgesic potential of the drugs at the dose that appears ineffective compared to other analgesic agents. Hence, peripheral pathway of antinociceptive can be measured by this model. The previous investigations had shown that acetic acid produced constrictions of abdominal muscles by stimulating the pain receptors to release the prostaglandins peripherally particularly PGE2-alpha and PGF2-alpha (Deraedt et al., 1980).

Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) provide protection against pain induction by inhibiting the prostaglandins (Meek et al., 2010). Conventional use of medicinal agents like NSAIDs to treat inflammation and analgesia, studies showed adverse effects of drugs like allergic reactions, gastric problems and other symptoms of analgesic abuses (Santangelo et al., 2007). Multiple resistance of medicine has developed in both the human and plant pathogenic organisms due to the inappropriate use of commercial medicines for the remedy of the infectious diseases (Rajeswari, 2015). Therefore, the mechanism of pain inhibition through C. sinensis L. seed and leaf extracts is unknown; the possible relief in pain symptoms is attributed to the inhibition of prostaglandins by these extracts. Current findings of inhibiting the last phase of inflammation and protecting the mice from visceral pain indicated that peripheral inhibition
of prostaglandins is a possible mechanism by which the extract induced pharmacological activities (Tatiya et al., 2017).

CONCLUSIONS

The objective of the phytochemical screening and antimicrobial activity was to standardize the natural medicinal plant. Any part of the plant can be used for pharmacological evaluation prior to preclinical to clinical trials. The phytochemical screening of all plant parts with different solvents as well as FTIR analysis of different morphological parts of powdered crude drug played important role in the determination of different phytochemicals present in the plant. Our results suggested that C. sinensis L. could be a source of potential antimicrobial agent which varies with the part of the plant. Also, analgesic and anti-inflammatory activity of C sinensis L. seed and leaf extracts in animal model demonstrated promising results. The findings reported highlights the fact that high dose of both C. sinensis L. seed and leaf extracts are safe for consumption with analgesic and anti-inflamatory potentials compared to standard drug group.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20210815170852

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Phytochemical and Pharmacological Potential of *Camellia sinensis* L.

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**Supplementary Table I. Percentage yield of root, stem, leaf and seed of *C. sinensis* L. with different solvents.**

| Plant part | Petroleum ether | Acetone | Ethanol | Water |
|------------|-----------------|---------|---------|-------|
| Root       | 5.0             | 2.9     | 1.0     | 1.0   |
| Stem       | 10.0            | 3.0     | 2.0     | 2.0   |
| Leaf       | 6.0             | 5.3     | 12.0    | 1.0   |
| Seed       | 12.0            | 10.9    | 20.0    | 1.0   |

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**Supplementary Table II. Phytochemical analysis of petroleum ether extracts of various parts of *C. sinensis* L.**

| Phytochemicals | Test reagents | Root | Stem | Leaf | Seed |
|----------------|---------------|------|------|------|------|
| Primary metabolites | | | | | |
| Carbohydrates | Benedict’s test | ++ | +++ | ++ | ++ |
| | Molisch’s test | ++ | ++ | ++ | ++ |
| Proteins | Xanthoproteic test | + | + | + | - |
| Fats and fixed oils | Stain test | ++ | - | + | +++ |
| Secondary metabolites | | | | | |
| Alkaloids | Dragendorff’s test | +++ | + | - | + |
| Glycosides | Fehling’s test | +++ | +++ | ++ | ++ |
| Saponins | Froth formation test | ++ | ++ | - | +++ |
| Tannins | Ferric chloride test | - | ++ | +++ | - |
| | Gelatin test | + | + | + | +++ |
| Resins | Acetone water test | +++ | +++ | +++ | +++ |
| Flavonoids | Lead acetate test | - | ++ | +++ | +++ |
| Lignin | Saffranine test | - | - | - | - |
| Tri-terpenoids | Salkowski test | ++ | +++ | +++ | - |
| Steroids | Vanillin-H$_2$SO$_4$ test | - | - | - | - |

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0030-8923/2022/0001-0001 $ 9.00/0

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Supplementary Table III. Phytochemical analysis of acetone extract of various parts of *C. sinensis* L.

| Phytochemicals       | Test reagents           | Root | Stem | Leave | Seed |
|----------------------|-------------------------|------|------|-------|------|
| Primary metabolites  |                         |      |      |       |      |
| Carbohydrates        | Benedict’s test         | -    | +    | -     | +++  |
|                      | Molisch’s test          | -    | +    | -     | ++   |
| Proteins             | Xanthoproteic test      | -    | -    | -     | -    |
| Fats and fixed oils  | Stain test              | -    | +    | +     | +++  |
| Secondary metabolites|                         |      |      |       |      |
| Alkaloids            | Dragendroff’s test      | -    | ++   | +     | -    |
| Glycosides           | Fehling’s test          | +++  | +++  | ++    | -    |
| Saponins             | Froth formation test    | +    | ++   | +++   | +++  |
| Tannins              | Ferric chloride test    | +    | +++  | +++   | -    |
|                      | Gelatin test            | -    | -    | +     | -    |
| Resins               | Acetone water test      | +    | +    | +++   | +    |
| Flavonoids           | Lead acetate test       | -    | ++   | +     | +    |
| Lignin               | Saffranine test         | ++   | +++  | +++   | ++   |
| Tri-terpenoids       | Salkowski test          | -    | ++   | +     | +    |
| Steroids             | Vanillin-H$_2$SO$_4$ test | + | ++ | + | + |

+, Slightly positive; +, Positive; +++, Strongly positive; -, Negative

Supplementary Table IV. Phytochemical analysis of ethanolic extracts of various parts of *C. sinensis* L.

| Phytochemicals       | Test reagents           | Root | Stem | Leave | Seed |
|----------------------|-------------------------|------|------|-------|------|
| Primary metabolites  |                         |      |      |       |      |
| Carbohydrates        | Benedict’s test         | ++   | ++   | -     | -    |
|                      | Molisch’s test          | +    | +    | +     | +    |
| Proteins             | Xanthoproteic test      | +    | +    | -     | -    |
| Fats and fixed oils  | Stain test              | -    | -    | -     | -    |
| Secondary metabolites|                         |      |      |       |      |
| Alkaloids            | Dragendroff’s test      | ++   | +    | -     | -    |
| Glycosides           | Fehling’s test          | +++  | ++   | -     | -    |
| Saponins             | Froth formation test    | ++   | -    | +     | -    |
| Tannins              | Ferric chloride test    | +++  | ++   | +     | +++  |
|                      | Gelatin test            | ++   | +++  | -     | -    |
| Resins               | Acetone water test      | +    | -    | ++    | +    |
| Flavonoids           | Lead acetate test       | ++   | +    | ++    | -    |
| Lignin               | Saffranine test         | -    | -    | +     | ++   |
| Tri-terpenoids       | Salkowski test          | ++   | +    | -     | +    |
| Steroids             | Vanillin-H$_2$SO$_4$ test | - | - | + | + |

+, Slightly positive; +, Positive; +++, Strongly positive; -, Negative

Supplementary Table V. Phytochemical analysis of water extract of various parts of *C. sinensis* L.

| Phytochemicals       | Test reagents           | Root | Stem | Leave | Seed |
|----------------------|-------------------------|------|------|-------|------|
| Primary metabolites  |                         |      |      |       |      |
| Carbohydrates        | Benedict’s test         | -    | +    | -     | -    |
|                      | Molisch’s test          | -    | -    | +     | -    |
| Proteins             | Xanthoproteic test      | ++   | +    | +     | +    |
| Fats and fixed oils  | Stain test              | -    | -    | -     | -    |
| Secondary metabolites|                         |      |      |       |      |
| Alkaloids            | Dragendroff’s test      | -    | -    | -     | -    |
| Glycosides           | Fehling’s test          | -    | +    | -     | -    |
| Saponins             | Froth formation test    | -    | +    | -     | -    |
| Tannins              | Ferric chloride test    | -    | +    | -     | -    |
|                      | Gelatin test            | -    | +    | -     | -    |
| Resins               | Acetone water test      | -    | -    | -     | -    |
| Flavonoids           | Lead acetate test       | -    | -    | -     | -    |
| Lignin               | Saffranine test         | -    | -    | -     | -    |
| Tri-terpenoids       | Salkowski test          | -    | -    | -     | -    |
| Steroids             | Vanillin-H$_2$SO$_4$ test | - | - | + | + |

+, Slightly positive; +, Positive; +++, Strongly positive; -, Negative