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# Phytochemical and antimicrobial screening of medicinal plants for the treatment of acne

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Extracts of *Camellia sinensis* Linn. leaves, *Glycyrrhiza glabra* Linn. roots and rhizome and *Calendula officinalis* Linn. flowers were screened for their *in vitro* antimicrobial activity using agar disc diffusion method. The antimicrobial activity of petroleum ether, dichloromethane and methanolic extracts of different parts of these plants were studied against acne causing bacteria, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 2639) and *Propionibacterium acnes* (MTCC *1951). Methanolic extract of *C. sinensis* leaves possessed highest antibacterial activity against *S. epidermidis*. Lowest minimum inhibitory concentration (0.625 mg/mL) and minimum bactericidal concentration (2.5 mg/mL) against *S. epidermidis* were also observed for methanolic extract of *C. sinensis* leaves. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides and terpenoids which indicates that these phytoconstituents may be responsible for their anti-acne activity.

**Keywords:** Antimicrobial activity, *Calendula officinalis, Camellia sinensis*, Disc diffusion method, *Glycyrrhiza glabra,* Phytochemical screening.

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## Introduction

*Camellia sinensis* Linn. (Family-Theaceae) known as green tea, is the second most common beverage consumed worldwide next to water[1]. It is an evergreen shrub or tree which is mainly cultivated in India and China. Three main varieties of tea have been reported namely green, black, and oolong. Green tea is made from unfermented leaves and contains the highest concentration of powerful antioxidants. Green tea has a number of pharmacological activities such as anticancer, lipid lowering, neuromuscular blocking action, immunomodulatory effect, antiviral, anti-bacterial[2], antispasmodic, antioxidant[3], anti-inflammatory[4], etc. A large number of phytoconstituents like alkaloids (caffeine, theobromine), proteins, enzymes, carbohydrates, lipids, polyphenols, catechins (epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate), carbohydrates, tannins, vitamins and minerals have been reported to be present in this plant[5].

*Glycyrrhiza glabra* Linn. (Family-Fabaceae) known as liquorice, has been used in medicines for more than 4000 years now. It is native to central and south west Asia, Mediterranean basin of Africa, South Europe and India. Various species of liquorice have been reported namely *Glycyrrhiza inflata* Fisch., *Glycyrrhiza uralensis* Fisch. and *Liquiritia officinalis* Moench[6]. In the traditional system of medicine, liquorice is used as a demulcent, anti-tussive, laxative, sweetener, diuretic, antiarthritic, antibacterial[7], anti-inflammatory[8], anti-acne[9], aphrodisiac, estrogenic, antioxidant, antineoplastic, anticholinergic and antiulcer[10]. Phytoconstituents isolated from this plant are glycyrrhizin, glycyrrhizinic acid, glabrin A and B, glycyrrhetol, glabrolide, isoglabrolide, isoflavones, coumarins, triterpene sterols liquiritin, isoliquiritin, flavones, chalcones and isoflavonoids, such as glabridin[7-11].

*Calendula officinalis* Linn. (Family-Asteraceae) known as Pot marigold is an important medicinal plant for the indigenous people of India, Europe, US and China. Its leaves and flowers have been reported to possess many pharmacological activities which include antioxidant, anti-inflammatory, antibacterial and antiviral activities[12-14]. Phytoconstituents isolated from this plant are sitosterols, stigmasterols, $\psi$-taraxasterol, lupeol, faradiol-3-*O*-palmitate,

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faradiol-3-*O*-myristate, faradiol-3-*O*-laurate, quercetin, isorhamnetin, isoquercetin, calendoflaside, α-tocopherol and essential oil rich in monoterpenes and sesquiterpenes[15].

Since the existing literature exhibits only a limited research concerning the utilization of these extracts in the treatment of acne, an attempt was made to evaluate the antimicrobial activity of petroleum ether, dichloromethane and methanolic extracts of leaves, roots, stolons and flowers of these plants, respectively by agar diffusion and broth microdilution methods.

## Materials and Methods

### Plant materials

Samples of *C. sinensis* leaves, *G. glabra* roots and stolons and *C. officinalis* flowers were collected from medicinal gardens and authorized herbal stores in Delhi and authenticated by NISCAIR, Pusa Campus, New Delhi with voucher specimen (NISCAIR/RHM/ consult/2008-09/978/09) and have been preserved in our department for the future reference.

### Extraction and preliminary phytochemical screening

Shade-dried plant leaves, roots, stolons and flowers (200 g) were pulverized separately and subjected to sequential solvent extraction by continuous hot extraction (soxhlet) method. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (PE), dichloromethane(DCM) and methanol(ME) and the extracts were abbreviated as: *Camellia sinensis* (CSPE, CSDCM, and CSME), *Glycyrrhiza glabra* (GGPE, GGDCM, GGME) and *Calendula officinalis* (COPE, CODCM, and COME). Every time, the marc was dried in air at room temperature and later used for extraction with other solvents. All the extracts were evaporated using a rotary evaporator and the percentage yield was thus recorded. Dried extracts were stored at 4°C in airtight containers for further studies. Concentrated extracts were subjected to various chemical tests in order to detect the presence of different phytoconstituents [16].

### Microorganism and media

Aerobic bacteria: *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 2639) and anaerobic bacteria: *Propionibacterium acnes* (MTCC*1951) were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh. Fresh cultures of the isolates of aerobic and anaerobic bacteria were suspended in nutrient broth and reinforced clostridium medium, respectively. *S. aureus* and *S. epidermidis* cultures were incubated for 24 h at 37 and 30°C, respectively. *P. acnes* culture was incubated in an anaerobic chamber at 37°C consisting of 10% $CO_2$, 10% $H_2$ and 80% $N_2$ for 48 h.

### Antimicrobial screening using disc diffusion method

Antibacterial activity of extracts was tested using agar disc diffusion method[17]. Fresh culture suspension (100 µl) of test bacteria was evenly spread on nutrient agar and reinforced clostridial agar plates. The concentration of cultures was $5 \times 10^5$ CFU/mL. For screening, 6 mm diam. filter paper disc, impregnated with 20 µl of extract solution equivalent to 0.2 mg of extract was placed on the surface of inoculated media agar plates. Incubation was done at 37°C or 30°C for 24 and 48 h depending upon the type of bacteria under optimum conditions. Clear zones of inhibition were measured in mm, including the diameter of disc. Zone measuring 10 mm or more was considered as effective against test organisms. Clindamycin (10 µg/disc) was used as positive control and the respective solvents, which were used for extraction, served as negative control.

### Minimum inhibitory concentrations using microdilution method

Minimum inhibitory concentration (MIC) of active methanolic extracts (AME) was studied by using broth microdilution method with slight modify-cations[18]. Extracts were dissolved in (DMSO) dimethyl sulfoxide (10% of total volume) and two fold dilutions were done using pre-sterilized culture broth to give final concentrations ranging from 5-0.078 mg/ml. 100 µl of each dilution was distributed in 96 well plates. Sterility control (sterilized nutrient broth) and growth control (culture broth with DMSO) were also set up. Each test and growth control well was inoculated with 5 µl of a bacterial suspension ($5x10^5$ CFU/ml). All experiments were performed in triplicate and the microdilution plates were incubated under optimum conditions. Bacterial growth was detected after the addition of 20 µl of 70% alcoholic solution of INT (0.5 mg/ml) into each well followed by incubation for 30 minutes. Colour change from yellow to purple indicated the presence of microbial growth.

### Minimum bactericidal concentration determination

The minimum bactericidal concentration (MBC) is defined as the lowest concentration of a compound

that kills the microorganism. The MBCs of the extracts were determined by plating 10 µl of samples from each MIC well without visible growth onto culture media plates[19]. Following the incubation for optimum period, the plates were examined for colony growth and MBCs were recorded.

## Results and Discussion

In present investigation, percentage yield of nine extracts indicated that GGME showed highest percentage yield (20.11) followed by COME (11.22) and CSME (9.55) (Figure 1). Phytoche ical screening showed the presence of the following phytoconstituents: *C. sinensis* (alkaloids, flavonoids, terpenoids and tannins), *G. glabra* (carbohydrate, glycosides, flavonoids, saponins, terpenes and sterol) and *C. officinalis* (flavonoids, saponins and terpenoids). *In vitro* antimicrobial screening using clindamycin phosphate as a positive control clearly indicated that CSME, GGME and COME show promising antimicrobial activity against all the three organisms (Table 1). It was observed that all the extracts of *C. sinensis* and *G. glabra* showed significant antimicrobial activity against test organisms except GGPE which did not exhibit antimicrobial activity against *S. epidermidis* (Figures 2-4).

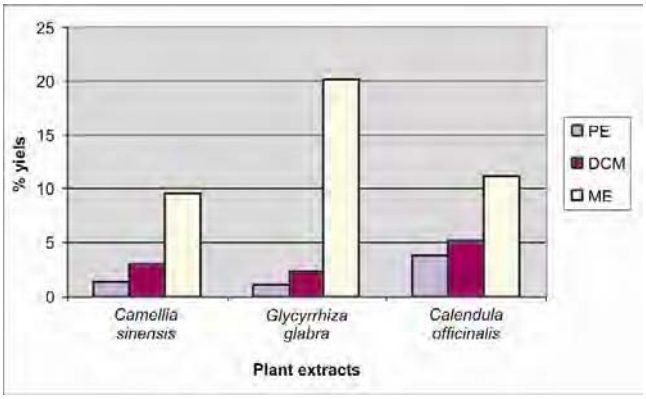


Fig. 1—Percentage yield of plant extracts

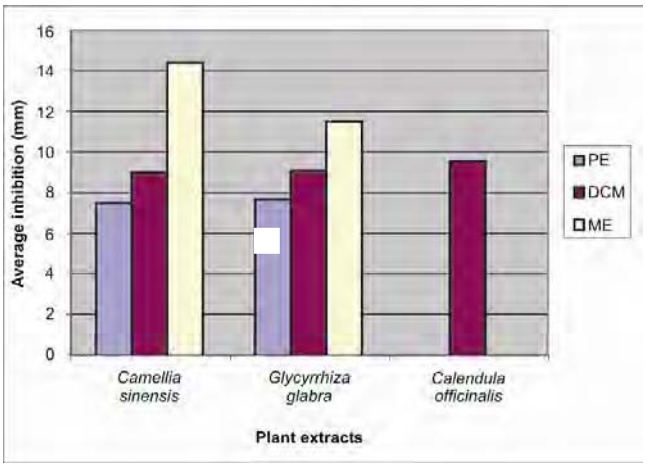


Fig. 2—Antimicrobial activity of plant extracts against *S.aureus*

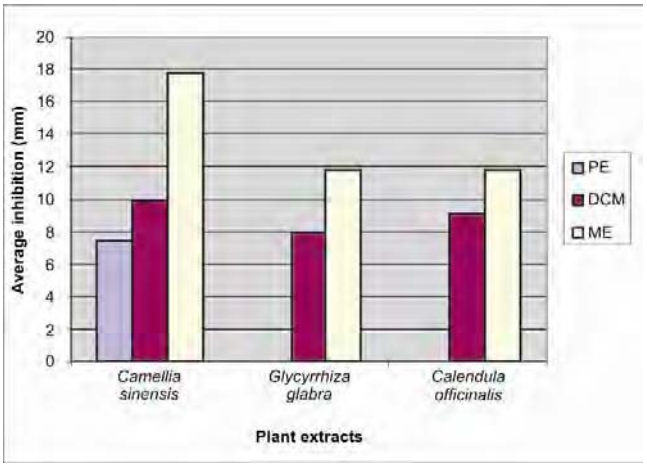


Fig. 3—Antimicrobial activity of plant extracts against *S. epidermidis*

Table 1—Antimicrobial screening of plants against *S. aureus* (MTCC 96), *S. epidermidis* MTCC 2639) and *P. acnes* (MTCC *1951) using disc diffusion method.

| | Zone of inhibition of extracts in mm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus** | | | *S. epidermidis** | | | *P. acnes** | | |
| | PE | DCM | ME | PE | DCM | ME | PE | DCM | ME |
| *Camellia sinensis* (Le) | 7.5±0.28 | 9.0±0.06 | 14.4±0.27 | 7.4±0.05 | 10.0±0.06 | 17.8±0.16 | 7.6±0.18 | 7.8±0.1 | 13.8±0.2 |
| *Glycyrrhiza glabra* (R&S) | 7.66±0.16 | 9.06±0.06 | 11.5±0.28 | NA | 8.0±0.12 | 11.8±0.15 | 7.1±0.05 | 13±0.05 | 13.9±0.15 |
| *Calendula officinalis*(Fl) | NA | 9.06±0.12 | NA | NA | 9.06±0.17 | 11.8±0.15 | 9.13±0.08 | NA | 12.4±0.18 |
| Clindamycin phosphate | 14.94±0.08 | | | 18±0.11 | | | 18±0.05 | | |

Le = Leaves; R & St = Roots and Stolons; Fl = Flowers; PE = Petroleum ether extract; DCM = Dichloromethane extract; ME = Methanolic extract; NA = No antibacterial activity. Values are Mean ± SEM (mm) of three measurements; *P< 0.05.

Table 2: Minimum inhibitory and minimum bactericidal concentrations of AMEs and Clindamycin

| Active methanolic extracts | *S. aureus* (MTCC 96) | | *S. epidermidis* (MTCC 2639) | | *P. acnes* (MTCC*1951) | |
|---|---|---|---|---|---|---|
| | MIC (mg/ml) | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) |
| CSME | 1.25 | 2.5 | 0.625 | 2.5 | 1.25 | 2.5 |
| GGME | 2.5 | >5 | 2.5 | 5 | 1.25 | 5 |
| COME | NA | NA | 2.5 | >5 | 2.5 | 5 |
| Clindamycin | 0.312 | 0.63 | 0.078 | 0.156 | 0.078 | 0.156 |

CSME = Methanolic extract of *Camellia sinensis*; GGME = Methanolic extract of *Glycyrrhiza glabra*; COME = Methanolic extract of *Calendula officinalis*; NA = No antibacterial activity. Results are average of three measurements.

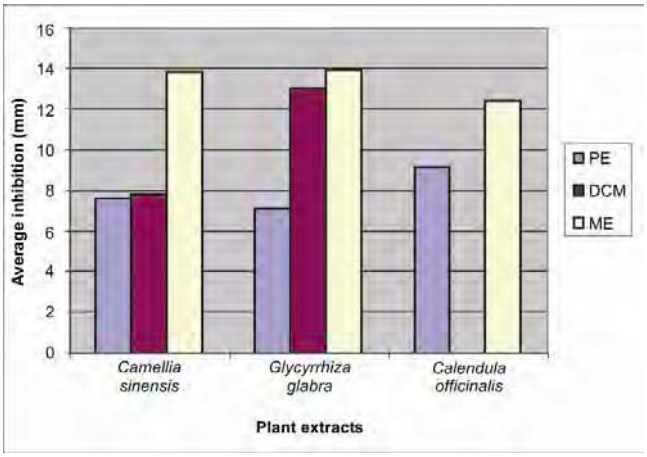


Fig. 4—Antimicrobial activity of plant extracts against *P. acnes*

CODCM and COME were found to be significantly active against *S. epidermidis,* however, they did not show inhibitory activity against *S. aureus* and *P. acnes*. Highest zone of inhibition 17.8±0.016 mm was observed for CSME against *S. epidermidis.* The results, after preliminary antimicrobial screening, revealed that active methanolic extracts have potential in the treatment of acne hence, they were further evaluated for their MICs and MBCs .The lowest MICs against *S. epidermidis* (0.625 mg/ml), *S. aureus* (1.25 mg/ml) and *P. acnes* (1.25 mg/ml) were recorded in CSME (Table 2). Similarly lowest MBCs against *S. aureus* (2.5 mg/ml), *S. epidermidis* (2.5 mg/ml) and *P. acnes* (2.5 mg/ml) were also observed in CSME (Table 2).

Though there is lack of literature to provide evidence for the antimicrobial activity of these medicinal plants against acne causing bacteria, yet our findings could be based on the bactericidal action of epigallocatechin gallate and epicatechin in *C. sinensis*[20]. Antibacterial and anti-inflammatory effect of *G. glabra* is attributed due to the presence of glycyrrhizin and its hydrolysis product, glycyrrhetinic acid[8]. Presence of triterpenoids in *C. officinalis* is known to provide anti-inflammatory activity. It was also reported that esters of faradiol-3-myristic acid, faradiol-3-palmitic acid and 4-taraxasterol are the three most active compounds to reduce edema and the flavonoid, kaempferol, demonstrated antibacterial activity against *P. acnes*[21]. Hence, the antimicrobial activity of AMEs showed broad spectrum potential as the active compounds are concentrated more in ME fraction than in PE and DCM fractions which supports phytochemical screening.

## Conclusion

The results clearly indicated that scientific studies carried out on these medicinal plants, possessing traditional claims of effectiveness in skin disorders, provided fruitful results. Therefore, methanolic extracts of *C. sinensis*, *G. glabra* and *C. officinalis,* possessing broad-spectrum activity could be utilized in treating acne vulgaris and formulating anti-acne herbal products. The main focus of our work is on the anti-acne potential of herbs which we plan to study further with the ultimate objective of providing scientifically validated herbal remedies against acne. Further in this regard, characterization is required to determine the types of compounds responsible for the antibacterial activity.

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