# Phytochemical screening, GC-MS analysis and antimicrobial activity of *Sphagneticola calendulacea* L. leaf extracts

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## ABSTRACT

This study was designed based on phytochemical screening of aqueous and methanolic extracts of antimicrobial activity of *Sphagneticola calendulacea* L. leaves against clinical microbes. The preliminary phytochemical screening of the crude extract revealed the presence of alkaloids, flavonoids, carbohydrate, glycosides, steroids, tannins, terpenoids, phenols, protein and triterpenoids. The phytochemical constituents of methanolic extract were studied through gas chromatography-mass spectrometry (GC-MS). Totally 16 phytocompounds were identified which include sterols, fatty acid and their methyl esters. The presence of bioactive constituents are studied the antimicrobial activity by well diffusion method.

**Key words:** *Sphagneticola calendulacea*, Phytochemical screening, antimicrobial activity, GC-MS analysis.

## INTRODUCTION

In recent years, an increasing awareness focused about the importance of medicinal plant. Drugs from these plants are easily available inexpensive, safe, efficient, and rarely accompanied by side effects[1]. Plants which have been selected for medicinally effective drugs such as antibacterial active pharmacological agents or as precursors for chemicopharmaceutical hemisynthesis. There is also increase in the use of medicinal plants[2,3].

*Sphagneticola calendulacea* is a flowering plant species produces wedelolactone and Chinese perennial plant, is a tender spreading herbaceous and hairy herb, with the branches usually less than 50cm long, the leaves are used in curing grey hair and in promoting the growth of hair[4]. They are considered as tonic, alternative and useful in coughs, lephalalgia, skin disease and alopecia[5]. The juice of the leaves is much used as a shaft in lephalalgia[6]. A devotion of the fresh plant is also used for patching babies to prevent lichen tropices skin infections[7]. The fresh juice from the leaves of *S. calendulacea* is used by Ayurvedic, physicians in India to treat skin problems, infection inflammation fungi, abscesses[8]. Leaves and stems contain the diterpene (kaurenoic acid), eudesmanolidelactones and luteolin.

The structure of trilobolide-6-O-isobutyrate ($C_{23}H_{32}O_9$) were isolated from the flower of *Wedelia trilobata*, shows an eudesmanolide sesquiterpene skeleton constructed from the fusion of two cyclohexane rings and a lactone ring. Main bioactive sesquiterpene lactones, trilobolid-6-O-isobutyrates A and B were isolated by bioassay-guided fractionation from the leaves of *Wedelia trilobata*, together with known trilobolides 6-O-isobutyrate and 6-O-methacrylate[9,10]. A study of the n-hexane extract of *Wedelia trilobata* showed antibacterial activity against *Bacilus subtilis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Salmonella group* and *Shigella sonnei*. Study in mice on the analgesic activity of the ethanolic extracts of *W. trilobata*, *W. bilofra* and *E.alba* showed dose-dependent blocking of writhing response[11].

A biological screening of activity against Gram-positive and Gram-negative bacteria, yeasts, and fungi of crude extracts from *Wedelia trilobata* is reported[12]. The n-hexane extract showed antibacterial activity against *Bacillus subtilis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (Gram-positive bacteria); along with *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, and *Shigella sonnei* (Gram negative bacteria). None of the tested extracts showed biological activity against the yeasts such as *Candida albicans*, *Candida tropicalis* and *Rhodotorula rubra* or the fungus are *Aspergillus flavus*, *Aspergillus niger*, *Mucor sp* and *Trichophyton rubrum*[13].

## MATERIALS AND METHODS

### Collection of plant materials:

The fresh and healthy leaves of the plant *S. calendulacea* L. were collected from Herbal Garden, A.V.V.M. Sri pushpam college (Autonomous) poondi, Thanjavur, Tamil Nadu, during the month of December 2017. The collected plant was identified and authenticated by Dr.S.John britto, Principal Scientific Officer, St. Joseph's college, Trichirappalli, Tamil Nadu, India.

### Preparation of plant extracts (Soxhelt method)

One gram of powder leaves blended with 50 mL of different solvents separately (aqueous and methanol) for different periods with agitation at room temperature. After the extract were allowed to filtration by using a 0.45 Millipore filter paper. The filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. Finally the extracts were allowed to weighed and stored at -20°C till their usage in the different tests. The qualitative and quantitative analysis of alkaloids, flavonoids, carbohydrates, saponins, tannains, terpenoids, steroids, triterpenoids, protein, phenols and glycosides as followed by Harbone (1973) method.

### GC-MS Analysis

The bioactive compounds present in the methanolic extract were analysed by GC-MS analysis. The analysis was performed according to the GC-MS equipments by Shimadzu QP 2010: RTX - column type is 5ms, Restek Corp (30 m length). The injector and detector temperatures were both maintained at 2500C, while

operation temperature at 50-3000C. The column temperature was programmed at 50-1200C. With 40C increase per min which was maintained for 1 min. Then it was programmed at 120-3000C, with 60°C increase per min and held for 5 min, with retention time (Rt) totally 60 min. Helium was used as a carrier gas is 50-500 atomic mass unit (amu). The compounds of each plant extracts were identified by using computer searchers in commercial libraries of Wiley.

**Antimicrobial activity (Agar well-diffusion method)**

The determination of antimicrobial activity of test plant extract by Agar well-diffusion method were analyzed. The nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old broth culture of respective bacteria and fungi were determined agar wells (5mm diameter) made in each of these plates using sterile cork borer. About different solvents of plant leaves extracts of *S. calendulacea* added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions of the plates were incubated in an upright position at 37± 2°C for 24 hrs for bacterial and 28± 2°C for fungi. The organic solvents (aqueous and methanol) were acted as a negative control results and the presence or absence of inhibition zone measured. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone as negative. The diameters of the zones measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antimicrobial activity.

**RESULTS AND DISCUSSION**

In the present investigation suggested that the qualitative and quantitative analysis of aqueous and methanolic extracts of *S. calendulacea* resulted the preliminary phytochemicals of alkaloids, flavonoids, carbohydrates, saponins, tannins, terpenoids, steroids, triterpenoids, protein, phenols and glycosides were analysed (Table-1). The quantitative analyses of aqueous and methanolic extracts of *S. calendulacea* were resulted in table.2. The results showed the methanolic extract has maximum phytochemical quantity when compared with aqueous extract.

The antibacterial activity of aqueous extract of *S. calendulacea* were resulted the maximum zone inhibition of 22.0±0.17 mm at 100 µL for *B.subtilis* and methanolic leaf extract showed 8.57±1.20 mm at 100 µL for *E.coli*. The antifungal activity of aqueous extract of *S. calendulacea* were observed the maximum zone inhibition of 8.30±2.76 mm at 100 µL for *A. flavus* and methanolic extract showed better activity of 8.22±2.66 mm at 100 µL *F.spp*.

The isolation of total phytochemical compounds by using GC-MS can accurate identify with respective retention time (table-3). In the present study suggested that the isolation of phytochemical constitutents of *S. calendulacea* leaf has elicited 16 compounds such as Cyclopropane, 1-butyl-2-pentyl-, cis, 4-

Cyclopropylcarbonyloxydodecane, Dodecanal, Diethyl Phthalate, Methyl 3,5-di-t-butylsalicylate, Phthalic acid, isobutyl octyl ester, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Hexadecanoic acid, 15-methyl-, methyl ester, Dibutyl phthalate, n-Hexadecanoic acid, Di-n-octylphthalate, 11,14-Eicosadienoic acid, methyl ester, 7-Hexadecenoic acid, methyl ester, (Z)-, Cyclopentaneundecanoic acid, methyl ester and Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans are identified. These phytocomponents are responsible for the antioxidant, antimicrobial activities and anti-inflammatory activities.

**Table 1: Qualitative phytochemical analysis of *S. calendulacea* leaf extract**

| Phytochemical compounds | Aqueous | Methanol |
|---|---|---|
| Alkaloid | + | + |
| Carbohydrate | + | + |
| Flavonoids | ++ | + |
| Glycosides | _ | _ |
| Phenol | + | + |
| Protein | + | + |
| Saponin | ++ | + |
| Steroids | + | + |
| Tannin | ++ | + |
| Terpenoids | - | + |
| Triterpenoids | + | + |

**(++) strongly present,( +) present, (--) absent**

**Table 2: Quantitative phytochemical analysis of *S. calendulacea* leaf extract**

| Name of the compounds | Quantity (mg/g) | |
|---|---|---|
| | Aqueous | Methanol |
| Alkaloids | 0.21±0.07 | 0.26±0.08 |
| Carbohydrate | 0.28±0.10 | 0.22±0.12 |
| Flavonoids | 0.72±0.07 | 0.79±0.26 |
| Phenol | 0.53±0.16 | 0.59±0.19 |
| Protein | 0.23±0.12 | 0.34±0.07 |
| Steroids | 0.87±0.09 | 0.94±0.31 |
| Saponin | 0.75±0.23 | 0.81±0.27 |
| Tannin | 0.84±0.05 | 0.86±0.28 |
| Terpenoids | - | 0.66±0.22 |
| Triterpenoids | 0.62±0.22 | 0.56±0.16 |

Standard deviation ± error

**Table 3: Phytochemical screening of *S. calendulacea* leaf extract by GC-MS**

| S. no | Retention Time | Compound name | Molecular Weight | Molecular formula |
|---|---|---|---|---|
| 1 | 9.423 min, Scan: 1871 | Cyclopropane, 1-butyl-2-pentyl-, cis | 168 | CAS No. 74663-88-0, C12H24, |
| 2 | 14.860 min, Scan: 3455 | 4-Cyclopropylcarbonyloxydodecane | 254 | C16H30O2 |
| 3 | 15.361 min, Scan: 3601 | Dodecanal | 184 | CAS No. 112-54-9, C12H24O |
| 4 | 21.613 min, Scan: 5407 | Diethyl Phthalate | 222 | CAS No. 84-66-2, C12H14O4 |
| 5 | 22.210 min, Scan: 5579 | Methyl 3,5-di-t-butylsalicylate | 264 | CAS No. 15018-03-8, C16H24O3 |
| 6 | 28.177 min, Scan: 7313 | Phthalic acid, isobutyl octyl ester | 334 | C20H30O4 |
| 7 | 29.327 min, Scan: 7648 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 276 | CAS No. 82304-66-3, C17H24O3, |
| 8 | 29.578 min, Scan: 7721 | Hexadecanoic acid, 15-methyl-, methyl ester | 284 | CAS No. 6929-04-0, C18H36O2 |
| 9 | 30.179 min, Scan: 7896 | Dibutyl phthalate | 278 | CAS No. 84-74-2, C16H22O4, |
| 10 | 31.202 min, Scan: 8194 | n-Hexadecanoic acid | 256 | CAS No. 57-10-3, C16H32O2 |
| 11 | 32.033 min, Scan: 8436 | Di-n-octylphthalate | 390 | CAS No. 117-84-0, C24H38O |
| 12 | 32.826 min, Scan: 8667 | 11,14-Eicosadienoic acid, methyl ester | 322 | CAS No. 2463-02-7, C21H38O2 |
| 13 | 32.885 min, Scan: 8684 | 7-Hexadecenoic acid, methyl ester, (Z)- | 268 | CAS No. 56875-67-3, C17H32O2, |
| 14 | 33.424 min, Scan: 8841 | Cyclopentaneundecanoic acid, methyl ester | 268 | CAS No. 25779-85-5, C17H32O2 |
| 15 | 36.404 min, Scan: 9709 | Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans | 310 | CAS No. 42199-20-2, C20H38O2 |

**Table 4: Antibacterial activity of *S. calandulata* leaf extract against bacteria's**

| Name of the bacteria | Zone of inhibition (mm) | |
|---|---|---|
| | Aqueous | Methanol |
| *Bacillus subtilis* | 22.0±0.17 | 8.21±0.86 |
| *E.coli* | 9.33±0.22 | 8.57±1.20 |
| *Enterococcus aeromonas* | 21.8±0.23 | 8.23±0.75 |
| *Pseudomonas aeroginosa* | 06.2±0.12 | 5.22±0.26 |
| *S.aureus* | 11.8±0.45 | 8.33±0.65 |

Standard deviation ± error

**Table 5: Antifungal activity of *S. calandulata* leaf extract against fungus**

| Name of the fungi | Zone of inhibition (mm) | |
|---|---|---|
| | Aqueous | Methanol |
| *Aspergillus flavus* | 8.30±2.76 | 1.06±3.53 |
| *A.niger* | 6.62±2.20 | 4.50±1.55 |
| *A.terreus* | 3.51±1.16 | 2.52±0.83 |
| *Fusarium* sp | 4.23±1.33 | 8.22±2.66 |
| *Penicillium citrium* | 5.55±1.83 | 1.03±3.43 |

Standard deviation ± error

## CONCLUSION

Phytochemical investigation of *S. calandulata* showed the presence of alkaloids, flavonoids, carbohydrate, saponins, tannin, terpenoids, steroids, triterpenoids, protein, phenol and glycosides. The quantitative analysis of methanolic extract showed highest phytochemical values and the GC-MS analysis were resulted the totally 16 phytocompounds were identified from methanolic leaf extract of *S. calandulata*. These phytocomponents were exhibited the various biological activities such as antioxidant, antimicrobial and antiflammatory activities. The antimicrobial activity of both aqueous and methanolic extracts showed better activities. Thus the present investigation suggested that the *S. calandulata* is phytochemically preferable one.

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