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Research Article

Phytochemical analysis of the flowers of *Chrysanthemum indicum* L. and *Calendula officinalis*

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Article History	Abstract
Received on: 09-02-2021 Revised On : 04-03-2021 Accepted on : 11-03-2021	Plant extracts have been used as a source of medicines for a wide variety of human ailments. the . Phytochemical analysis of flowers of ornamental plants of <i>Chrysanthemum indicum</i> L. and <i>Calendula officinalis</i> are very useful in identifying new sources of therapeutically important compounds like saponins, terpenoids, flavonoids and steroids are present in the flower extract of <i>Chrysanthemum indicum</i> L and carbohydrates, flavonoids, terpenoids are present in the flower extract of <i>Calendula officinalis</i> . <i>Chrysanthemum indicum</i> L :Hindi :Guldaudi, Sanskrit : Bahupatrika, Odiya: Shevathi <i>Calendula officinalis</i> : English : Marigold ,Hindi : Genda.
Keywords: Phytochemical Analysis, flowers , <i>Chrysanthemum indicum</i> L, <i>Calendula officinalis</i> .	
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Introduction

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bio-active substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, anti-oxidants and phenolic compounds. Phytochemists study phytochemicals by first extracting and isolating compounds from the origin plant, followed by defining

their structure or testing in laboratory model systems, such as cell cultures, in vitro experiments, or in vivo studies using laboratory animals. Challenge in that field include isolating specific compounds and determining their structures, which are often complex, and identifying what specific phytochemical is primarily responsible for any given biological activity. Phytochemicals are chemicals produced by plants. These are the chemicals that plants use to defend themselves against disease [1,2].

Extraction methods involved in this project are Maceration, Percolation, Soxhlet Extraction, Supercritical fluid extraction, Microwave assisted extraction, Ultrasound assisted extraction, Accelerated solvent extraction. The formation of yellow colour indicated the presence of flavonoids while the brown colour formation indicated the presence alkaloids and terpenoids. The phenol content was maximum in roots (82.13 mg/gdw) followed by seed leave, stem and fruit.

The sugar content was highest in leaves (8.27 mg/gdw) followed by fruits, stem, root and seed. The protein content was maximum in fruits (55.59 mg/dgw) followed by seeds leaves, stem and root [3].

Chrysanthemum indicum L.



Scientific classification

Kingdom: Plantae
 Phylum : Tracheophyta
 Class : Angiosperms
 Clade : Asterids
 Order : Asterales
 Family : Astreaceae
 Genus : Chrysanthemum
 Species : C. Indicum L.

Uses

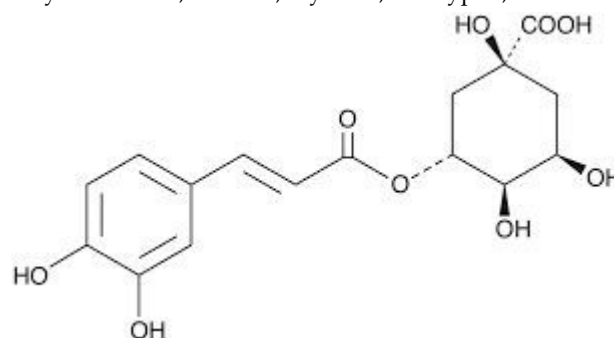
- The whole plant is antiphlogistic, blood tonic, depurative, febrifuge and vulnerary, dissipating heat, detoxifying, and dissipating blood stasis. In conjunction with black pepper it is used in the treatment of gonorrhoea and Hypertension.
- The flowers are aperient, bitter, hypotensive, stomachic and vasodilator.
- They contain the glycoside chrysanthemin that yields glucose and cyanidin on hydrolysis, together with stachydrine and an essential oil.
- They have an antibacterial action, inhibiting the growth of Staphylococcus, E. Coli, streptococcus, C. Diphtheriae, Bacillus dysenteria.
- The flowers are used in the treatment of furuncle, scrofula, deep-rooted boils, inflammation of the throat, eyes and cervix, eczema, itchiness of the skin .
- They have a rejuvenating effect when

used over a long period of time.

- An essential oil obtained from the plant contains chrysanthenone, this is active on the brain center affected by Parkinson's disease.

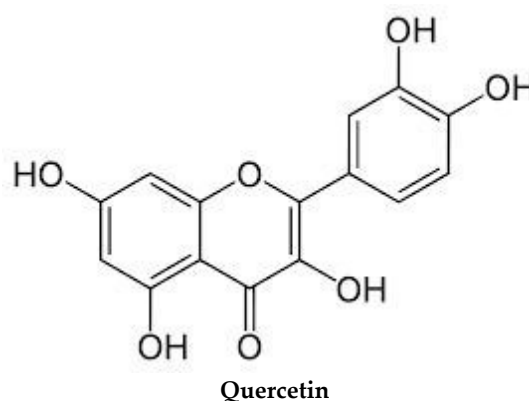
Phyto chemistry

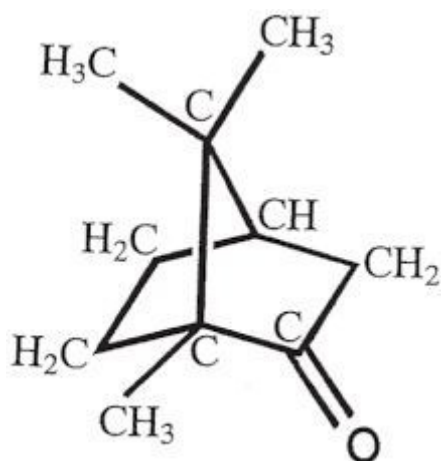
Phytochemicals are biologically active chemical compounds which are derived from plants. They have many health benefits for humans further than those attributed to macronutrients and Chrysanthemum spp. Flower contains octa-cosyl alcohol, β -sitosterol, lupeol, α -amyrin, daucosterol, ineupatorolide B, syringin, chlorogenic acid, petasiphenol, physcion, acacetin, eupatilin, quercetin, diosmetin, luteolin, apigenin, apigenin- 7-O- β -D-glucopyranoside, quercetin-3-O- β -D-glucopyranoside, luteolin-7-O- β -D-glucopyranoside, apigenin-7-O- β -D-neospheroside, and acacetin-7-O- β -D-glucoside. Most of the Chrysanthemum flowers contain anthocyanins, cyanidin 3-glucoside and cyanidin 3-(3''-malonoyl) glucoside and carotenoids: lutein, zeaxanthin, β -cryptoxanthin, 13-cis- β -carotene, α -carotene, trans- β -carotene, and 9-cis- β -carotene. The major volatile compounds present in the plants are camphor, α -pinene, chrysanthenone, safranal, myrcene, eucalyptol,



2,4,5,6,7,7ab-hexahydro-1H-indene, verbenone, β -phellandrene and camphene [3,4].

Chlorogenic acid





Camphor

Calendula officinalis



Scientific classification

Kingdom : Plantae
 Phylum : Tracheophyta
 Class : Angiosperms
 Clade : Asterids
 Order : Asterales
 Family : Astereaceae
 Genus : Calendula
 Species : C. Officinalis

Synonyms

- Calendula aurantiaca kotschy ex Boiss.
- Calendula eriocarpa DC.
- Calendula hydruntina (Fiori) Lanza
- Calendula prolifera Hort. ex Steud.
- Calendula x santamariae Font Quer
- Caltha officinalis (L.) Moench

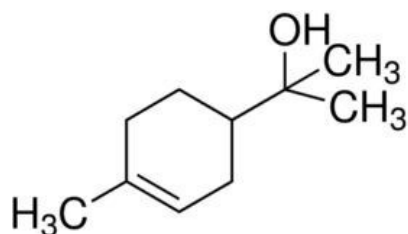
Uses

Calendula is an active ingredient in several diaper creams for babies. It is also used to make natural hand lotions and first-aid creams. It is useful in making a lotion for skin problems like eczema, psoriasis, acne, sunburn, wounds, athletes foot and ringworm. It can add to homemade DIY shampoo and foot soak a yellow dye obtained from the boiled flowers can also be used to add colour to hair.

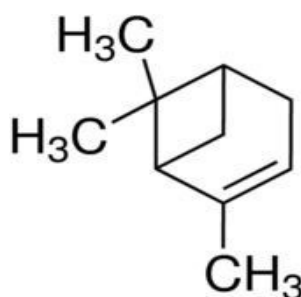
Phytochemistry

Identification of primary and secondary constituents has become the utmost important tool for the presence of active moiety. The phytochemical screening of Petroleum ether, Methanol and Ethanol extracts of calendula officinalis flowers. Petroleum extracts show the presence of fatty acids, ethanol extracts show the presence of triterpenes and sterols. Methanol extract shows saponins, phenolic substances. Various flavonoids have been isolated from the ethanol extract of the flowers of C. officinalis. They include quercetin, isorhamnetin, isoquercetin, isorhamnetin-3-O- β -D-glycoside, narcissin, calendoflaside, calendoflavoside, calendoflavobioside, rutin, isoquercitrin, neohesperidoside, isorhamnetin-3-Oneohesperidoside, isorhamnetin-3-O-2G - rhamnosyl rutinoside, isorhamnetin-3-Orutinoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside. Coumarins The ethanol extract of the inflorescence of the C. officinalis reported to contain coumarins - scopoletin, umbelliferone and esculetin. Quinones reported from C. officinalis were plastoquinone, phylloquinone, α -tocopherol in the chloroplast, ubiquinone, phylloquinone, atocopherol in mitochondria, and phylloquinone in the leaves. Volatile oil C. officinalis flowers contain maximum volatile oil at full flowering stage (0.97 %) and minimum during the preflowering stage (0.13 %). The composition also showed different patterns at different phases of vegetative cycles. Various monoterpenes and sesquiterpenes have been reported in the volatile oil : α -thujene, sabinene, β -pinenene, limonene, 1,8-cineol, p-cymene, trans- β -ocimene, γ -terpenene, δ -3-carene, nonanal, terpene-4-ol, 3-cylohexene-1-ol, α phellandrene, α -terpeneol, geraniol, carvacrol, bornyl acetate, sabinyl acetate, α cubebene, α -copaene, α -bourbonene, β cubebene, α -gurjunene, aromadendrene, β caryophyllene, α -ylangene, α -humulene, epibicyclo-sequiphellandrene, germacrene D, alloaromadendrene, β -saliene, calarene, muurolene, δ -cadinene, cadiene, α adinene, nerolidol, palustron, endobourbonene, oplopenone, α -cadinol, Tmuurolol

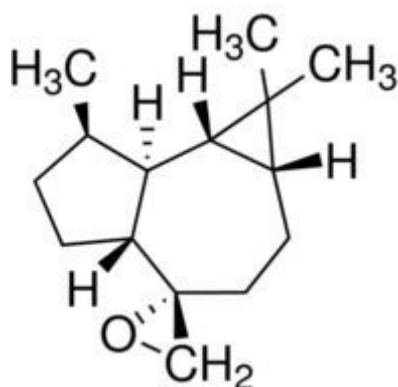
[5,6]. The essential oil was found to be rich in α -cadinene, α -cadinol, t-muurolol, limonene, and 1,8-cineol with p-cymene at lower levels at the post-flowering period.



α -Terpenol



β - pinenene



Aromadendrene

Methodology

Methodology of *Chrysanthemum indicum* L.

Collection of plant material

- The flowers of *Chrysanthemum indicum* L. were collected from Tagarapuvalasa market, Vizianagaram district, Andhra Pradesh, India in December 2019..

Preparation of extract

The collected flowers were washed with distilled water and dried in shade for 4-6 days. They were then coarsely powdered and extracted completely by cold maceration. The solvents used for maceration were petroleum ether, methanol and Ethanol (95%).

Procedure for Maceration

By soaking 77.4 g of each samples in 250ml of solvents (petroleum ether, methanol and ethanol) by cold maceration and allow them to stand with intermittent shaking for 3 days. The extracts were filtered using Whatman no 41 filter paper. The filters were collected and made up to knowm volume and stored in refrigerator at 4°C.



Qualitative Analysis of Phytochemical constituents

The flower extracts (ethanol, methanol, petroleum ether) were subjected to preliminary phytochemical studies to qualitatively analyze the active components present in them.

Phytochemical evaluation

1. Test for Alkaloids

The extracts of flower were warmed separately with 2% H₂SO₄ for 2 minutes. It was filtered and few drops of following reagents were added, which indicated the presence of alkaloids. Dragendroff's reagent: A red precipitation indicated the positive test. Mayer's reagent: A creamy white colored indicated the positive test. Picric acid: A yellow precipitation indicates the positive test.

2. Test for Flavonoids

A small quantity of the extract was heated with 10ml of ethyl acetate in boiling water for 3mins. The mixture was filtered and the filtrates were used for the following tests. The filtrate was shaken with 1ml of dil. Ammonia solution(1%). The layers were allowed to separate. A yellow coloration was observed in ammonia layer indicating the presence of flavonoid. The filtrate was shaken with 1ml of 1% Ammonium Chloride solution, where light yellow colour

was observed. It indicated the presence of flavonoid.

3. Test for carbohydrates

The extracts were shaken vigorously with water and filtered. A few drops of molisch's reagent was added to the aqueous filter, followed by vigorous shaking again. Concentrated H_2SO_4 (1ml) was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive test.

4. Test for Saponins

A small quantity of different extracts was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable foam, which indicated positive test.

5. Test for steroids

2 ml of Acetic anhydride and 2ml H_2SO_4 were added to the extracts. The color changed from violet to blue or green, which indicated the presence of steroids.

6. Test for Anthraquinone glycosides (Borntrager's test)

Dil. H_2SO_4 was added to the extracts and boiled. Then filtered and cooled. To the cold filtrate, 3 ml of benzene was added and mixed. The benzene layer was separated and ammonia (2ml) solution was added to it. A rose pink to red color in ammoniacal layer was observed, which indicated positive test.

7. Test for cardiac glycosides (Legal's test)

To each extract, 1ml of pyridine and 1ml of sodium nitroprusside solution were added and observed. A deep red color was observed indicating the positive test.

8. Test for terpenoids (salkowski test)

Each extract was mixed with 2ml of chloroform and then concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration at the interface indicated positive result for the presence of terpenoids.

9. Test for gum and mucilages:

Each extract was dissolved in 10ml of distilled water and 25 ml of absolute alcohol was added to it with constant stirring. White or cloudy precipitate indicated the presence of gum and mucilages.

10. Test for Amino acids and proteins

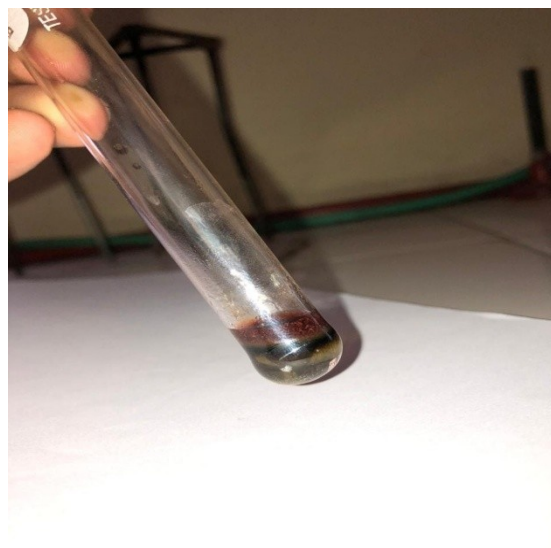
Each extract was dissolved in 10 ml of distilled water and the filtrate was subjected to test the presence of proteins and amino acids.

a) Biuret Test

2 ml filtrate was treated with one drop of 2% Copper Sulphate solution and then 1ml of ethanol (95%) was added to it followed by excess potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

b) Ninhydrin Test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added to 2ml of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.



Results

Chrysanthemum

indicum L.

- Initial weight of dried flowers = 231g
- Weight of the powdered flowers = 130g
- Weight of the powder used for the experiment = 77.
- Weight of the petroleum ether extract = 1.1g
- Weight of the methanol extract = 3.6g
- Weight of ethanol extract = 2.9



Phytochemical screening results

S.no	Phytochemical constituents	Petroleum ether	Methanol	Ethanol
1	Carbohydrates	-	+	+
2	Saponins	+	+	+
3	Alkaloids	-	+	+
4	Cardiac glycosides	-	-	+
5	Amino acids and proteins	-	-	-
6	Gums and mucilage	-	-	-
7	Terpenoids	+	+	+
8	Flavonoids	+	+	+
9	Steroids	+	+	+

'+' indicates presence '-' indicates absence

Methodology of calendula officinalis

Collection of plant material

The flowers of *Calendula officinalis* were collected from Brahmapur, Ganjam district, Odisha, India in November 2020.

Preparation of extract

The collected flowers are washed with distilled water and dried in shade for 4-6 days. They were then coarsely powdered and extracted completely by cold maceration. The solvents used for maceration were petroleum ether, methanol and Ethanol(95%).

Procedure for Maceration of Candula Officinalis

By soaking 133.3 g of each samples in 400ml of solvents (petroleum ether, methanol and ethanol) by cold maceration and allow them to stand with intermittent

shaking for 3 days. The extracts were filtered using Whatman no 41 filter paper. The filters were collected and made up to knowm volume and stored in refrigerator at 4°C. Qualitative Analysis of Phytochemical constituents : The flower extracts (petroleum ether, methanol and ethanol) were subjected to preliminary phytochemical studies to qualitatively analyze the active components present in them.

Results

Calendula officinalis

- Initial weight of dried flowers
- Weight of the powdered flowers
- Weight of the powder used for the experiment = 133.3g
- Weight of the petroleum ether extract = 0.9g
- Weight of the methanol extract
- Weight of ethanol extract

S.no	Phytochemical constituents	Petroleum ether	Methanol	Ethanol
1	Carbohydrates	+	+	+
2	Saponins	-	+	-
3	Alkaloids	-	+	-
4	Cardiac glycosides	-	-	-
5	Amino acids and proteins	-	+	+
6	Gums and mucilage	-	-	-
7	Terpenoids	+	+	+
8	Flavonoids	+	+	+
9	Steroids	-	-	-

'+' indicates presence '-' indicates absence

Discussion

The medicinal value of these plants lies in some chemical substances that have a definite physiological action on body [1,2]. The most important of these bioactive constituents of the plants are alkaloids, terpenoids flavonoids, steroids, cardiac glycosides and protein compounds. Results showed that the compounds like saponins, terpenoids, flavonoids and steroids are present in the flower extract of *Chrysanthemum indicum* L. Results showed that the compounds like carbohydrates, flavonoids, terpenoids

are present in the flower extract of *Calendula officinalis* [4-6].

Summary

The thesis consists of phytochemical investigation of some potent indigenous medicinal plants of traditional use namely *Chrysanthemum indicum* L. and *Calendula officinalis*(flowers).

Conclusion

The plants studied here can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and claims about the therapeutic values of these plants as curative agent. We therefore, suggests further the isolation, purification and characterization of the bioactive compounds of the flower part of *Chrysanthemum indicum* L. and *Calendula officinalis* with a view to obtain some useful chemotherapeutic agents.

Author Contribution

All authors Contributed Equally.

Conflict of Intrest

The authors declares no conflict of Intrest.

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