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PHYTOCHEMICAL SCREENING OF *CALENDULA OFFICINALIS LINN* FLOWER EXTRACT

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ABSTRACT

Goal of the present investigation to screen the numerous phyto-chemicals from the hydro-ethanolic extracts of flowers of *Calendula Officinalis Linn*. Pot Marigold is one of the most known synonyms, and belongs to the Asteraceae family subject for many chemical profiling and therapeutic assessments. Chemical investigations mainly focused on the existence of various compound classes. The flower which is reported with a various pharmacological and therapeutic functions encompassing antioxidant, anti-inflammatory, antifungal, antibacterial, and antiviral properties, as well as reported with tumor reducing potential, therapeutic properties include analgesic (pain-relieving), anthelmintic (anti-parasitic), antibacterial, antiemetic (prevents nausea), antifungal, anti-inflammatory, antipyretic (fever-reducing), antiseptic, antispasmodic (relieves muscle spasms), antiviral, astringent, bitter tonic, candidicidal (anti-Candida), cardiotonic (heart-strengthening), carminative (relieves gas), cholagogue (promotes bile flow), and dermagenic (skin-healing). etc. In this research work, we have focused on preliminary qualitative phytochemical screening and were found to contain various secondary metabolites by referring standard procedures and analytical technique like carbohydrates, proteins, alkaloids, amino acids, glycosides and saponins compounds are present. These elements having potential application as a medicinal agent.

KEYWORDS: *Calendula Officinalis Linn*, alkaloids, glycosides, extract, and phytochemical.

INTRODUCTION

Additionally, Calendula officinalis Linn has been termed by an extensive list of synonyms in our traditional health care system, that include Pot Marigold (English), Ekhwanasfar and Atherionmakhzani known by various names across different languages, including: Arabic: (name in Arabic, if needed), English: African marigold, Calendula, Common marigold, Garden marigold, Marigold, Chinese: Chin Chan Ts'ao,"[1] It is a valuable clinical botanical botanical species that is used to cure a kind of illnesses. Asteraceae is a well-known family to which it belongs.[2] The height of 30 to 60 cm is characteristic of Calendula officinalis linn. It has a solid, hairy, angular stem; lower, spatulate leaves that are measuring approximately 10–20 cm in length and 1–4 cm in width; taller, elongated, and tapering to a pointed tip (mucronate) leaves that are ranging from 4–7 cm in length, the outer epidermis' apical region features anomocytic stomata, along with covering and glandular trichomes. It contains elongated sclerenchyma cells and marginal flower heads that exhibit a bright yellow to orange hue. The oblong, spatulate corolla measures approximately 15–25 mm in length and about 3 mm in width. At its peak, the corolla of disc flowers is rounded, tridentate, and spans 1.5–2.5 cm in length with a diameter of 4–7 mm, while the tubular florets reach about 5 mm in length. The raw sienna powder derived from *Calendula officinalis* emits a distinct, fragrant aroma and has a mildly bitter taste.[3,4]

Chemical elements found in C. officinalis, such as plant phenolics, which contains lutein, quercetin etc., as well as triterpendiol esters and saponins. Orange blooms have high caroteniodes (mainly with rubixanthin and lycopene structures). Calendula officinalis phytochemical examination discovered that fatty acids had been detected in petroleum ether extracts while triterpens and sterols were identified in chloroform extracts. The water extract of Calendula officinalis contained tannins, phenolic compounds, and saponins, while the methanol extract comprised flavonoids, carbohydrates, amino acids, and saponins. Yet in another examination, tannin, carotenoids, steroids, and saponins have been identified in petroleum ether extract. Steroids, triterpens, and tannin Were observed in the chloroform extract, characterized by the presence of of alkaloids, flavonoids, and saponins has been proven by the solvent extract (ethanol). Plant-derived flavonoids and surfactant-like saponins were detected in the aqua-derived extract.[6,7,8]

Figure 1: *Calendula Officinalis Linn* flower.

The flower is said to have an extensive spectrum of pharmacological contribution, especially antiviral, antifungal, antibacterial, anti-inflammatory, and antioxidant capabilities. It also has the ability to diminish malignancies and trigger cytotoxicity. It shows analgesic, anthelmintic, possessing astringent, bitter, antifungal (candidicide), heart-strengthening (cardiotonic), digestive aid (carminative), bile-stimulating (cholagogue), skin-healing (dermagenic), sweat-inducing (diaphoretic), diuretic, blood-clotting (haemostatic), immune-boosting (immunostimulant), lymphatic-supporting, and uterine-contracting (uterotonic) propertiesantibacterial, antiemetic, antifungal, anti-inflammatory, antipyretic, antiseptic, anti-spasmodic, antiviral, astringent, cholagogue, anthelmintic, and vasodilator etc represent a few of its applications.[9,10] It is usually employed topically to heal open wounds, bleeding laceration wounds, and skin inflammations. Additionally, it is used to treat minor wounds like wind burns and razor burns. Inflammations of mucous membranes, peptic and duodenal ulcers, gastrointestinal tract spasms, intestinal and duodenal mucosa, dysmenorrhea (painful menstruation), particularly in women who are anxious or anemic, and splenic and hepatic inflammations are among its internal uses. Additionally, it is used as a rinse after tooth extractions.,[11,12] By considering all the aspects, the study is mainly aimed to analyze the botanical metabolites identified in the extract of *Calendula Officinalis Linn* by standered methods.

MATERIALS AND METHODS

I Materials

Collection of plant materials

Freshly collected blooms of the plant *Calendula Officinalis Linn.* were sourced from natural habitats in and around the local market of Belagavi Karnataka (India) which was identified

and authenticated from the voucher specimen accession number RMRC-1828. The harvested leaves were shade-dried, crushed into a coarse powder, and utilized for further analysis.

II Methods

Production of *Calendula officinalis* Linn. Flower Extract: Materials Required:Fresh *Calendula officinalis* Linn. flowers (20g), Ethanol (450 mL), Water (50 mL), Soxhlet apparatus, Rotary evaporator and Round-bottom flask. Procedure: Collection & Cleaning: Fresh *Calendula officinalis* flowers are collected and accurately weighed (20g). The flowers are washed thoroughly under flowing water twice to wash off any impurities. Drying: The cleaned flowers are shade-dried properly to retain their phytochemical properties. Extraction: The dried flowers are loaded into the Soxhlet apparatus. A hydro-ethanolic solvent mixture (450 mL ethanol + 50 mL water) is added. The Soxhlet extraction is carried out for 10 hours under continuous heating. Solvent Removal: After extraction, the obtained solution is transferred into a round-bottom flask. The ethanol recovered with the help of rotary evaporator, reducing extract to a semi-solid consistency. This hydro-ethanolic extract is now ready for phytochemical screening and further pharmacological studies.[13]

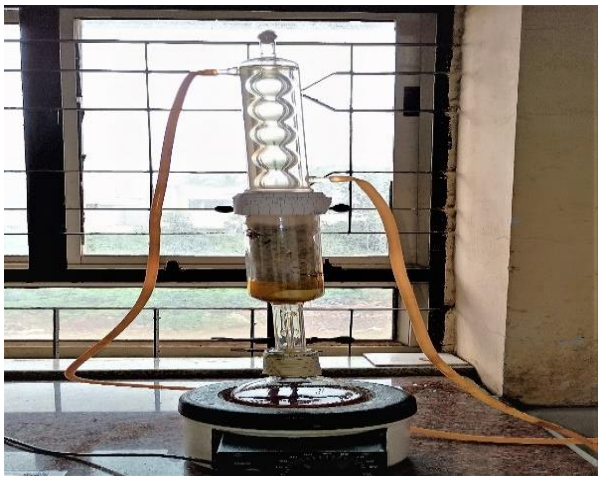

Figure 2: Soxhlet extraction apparatus.

B. Qualitative Phytochemical analysis: Qualitative bioactive compound screening of the plant *C. Officinalis* Bloom Isolate was performed using standard experimental method to determine the different phytochemicals which is existing in the flower extract.[14,15,16]

1. Test for carbohydrates

a. **Fehlings test:** Mix one mL of Fehling's solution A (copper sulfate) and 1 mL of Fehling's solution B (sodium potassium tartrate in sodium hydroxide). Boil the mixture

for 1 minute. Add an equal volume of the test solution (the sample being tested). Expose the mixture to heat using a boiling water bath for approximately 5–10 minutes. Check the intensity of colour difference: First, a yellow precipitate appears. Then, a brick-red precipitate forms, demonstrating the existence of Reactive sugars.

b. Molish test: pick 2 mL of the test solution in sterilized test tube. mix 2-3 drops of Molisch's reagent (α-naphthol in ethanol). Carefully add sulfuric acid (H_2SO_4) down the sides of the test tube and should not mixed during adding. notice for the appearance of ring with purple or violet colour between the two solutions.

c. Benedict's test: Take 5 mL of Benedict's solution mix around 8 drops of urine (test sample). Heat the mixture in a boiling water bath or over a flame 2 min. then Allow cool it. Observe colour change.

2. Test for proteins

a) Biuret test: measure 2 mL of the sample. sodium hydroxide (NaOH) solution added into it. 5–6 drops of copper sulfate ($CuSO_4$) solution introduce into the mixture. shake the mixture gently. Let the mixture sit for 4–5 minutes.Observe for a color change.

b) Million's test: 2–3 drops of Millon's reagent added to the sample then shake the contents to mix properly. Heat the test tube slightly if necessary. Observe for a colour change.

3. Test for alkaloids

a) Dragendroff's test: Dissolve the flower extract in chloroform. Evaporate the chloroform completely, leaving behind the residue. Add little drops of Dragendorff's reagent to the residue. Acidify the mixture (if necessary) using a few drops of dilute hydrochloric acid (HCl). Observe for any color change or precipitate formation.

b) Wagner's test: To the some drops of Wagner's reagent 3ml of extract, were introduced and noticed for reddish brownprecipitate.

c) Tannic acid test: A equal volumes of mixture hydro-ethanoic extract and tannic acid will give a buff-colored precipitate.

d) Mayer's test: 2-3 ml of filtrate with few drops of Mayer's reagent.

4. Test for flavonoids

a. Lead acetate test: Exactly 10 mg of the extract weighed. Add 10% lead acetate solution to the extract. The formation of a yellow precipitate confirms the presence of flavonoids**.**

b. Shinoda test: A mixture of 5ml of 95% ethanol/t-butyl alcohol, was mixed with 2ml extract and a few points of concentrated HCl solution added, followed by the addition of 0.5gm magnesium turnings, the appearance of orange, and pink, red to purple color.

5. Test for amino acids

a. Ninhydrin test: A solution containing 1% amino acid is prepared using distilled water. To this mixture, a few drops of a 2% ninhydrin solution are added. The test tube is then placed in a hot water bath for around 5 minutes. After heating, the appearance of a blue or violet color signifies the presence of amino acids, amines, or protein groups.

6. Test for glycosides

a) Killer Killiani test: Take the extract test solution in a cleaned test tube. Small portion of glacial acetic acid to the solution. Pour 2 mL of ferric chloride ($FeCl_3$) solution and mix gently. Carefully add concentrated sulfuric acid (H_2SO_4) from the sides of the test tube to create a layer separation. Observe the color changes in the two layers. Interpretation: Positive Result: The lower layer appears reddish-brown. The upper layer turns bluish-green. This color change indicates the presence of glycosides in the sample. Negative Result: No significant color change or layer separation suggests the absence of glycosides.

b) Legal's test: Around 3ml of *Calendula Officinalis Linn* flower extract were added to 1 ml of pyridine solution and 1 ml of sodium nitroprusside and observed for any color change from pink to red because that result shows the presence of glycosides.

c) Borntrager's test for anthraquinone glycosides: Take 1 mL of Calendula officinalis Linn flower extract in a test tube. Add a few drops of sulfuric acid (H_2SO_4) to the extract. Boil the mixture for a few minutes and then filter it. Allow the filtrate to cool. Add an equal volume of benzene or chloroform to the cold filtrate and shake the mixture well. Separate the organic solvent layer carefully. Add a few drops of ammonia (NH_3) solution to the separated organic solvent layer. Observe for a color change in the ammonia layer. Interpretation: Positive Result: The ammonia layer turns pink or red, indicating the presence of anthraquinone glycosides. Negative Result: No color change in the ammonia layer suggests the absence of anthraquinone glycosides.

7. Test for phenols

a. **Ferric chloride test**: Dissolve the small amount of extract in water and then gradually add a neutral ferric chloride solution, drop by drop. Observe any color changes like a red, blue, green, or purple color which indicates the presence of phenol.

8. Test for terpinoids

a. **Liebermann-Burchard test:** To 1 ml of the flower extract, chloroform and acetic anhydride were added, followed by addition of few drops of sulfuric acid (H_2SO_4). The presence of terpenoids can be indicated by the appearance of a dark green color.

RESULT AND DISCUSSION

The **preliminary phytochemical screening** of *Calendula officinalis* Linn. flower extract confirms the presence of several **bioactive compounds**, including: **Carbohydrates, Proteins, Amino acids, Glycosides, Flavonoids, Alkaloids, Tannins, Terpenoids, Quinones, Saponins**. The presence of these metabolites provides valuable insights into the **therapeutic potential** of *Calendula officinalis*. **Pharmacological Importance of Identified Compounds: Alkaloids**: Known for their **antispasmodic** and **antibacterial** activities. **Flavonoids**: Exhibit **antioxidant**, **anti-inflammatory**, **wound-healing**, and **anti-plaque** properties. **Saponins**: Possess **anthelmintic**, **hepatoprotective**, and **anti-inflammatory** effects. The **phytochemical screening data** in Table 1. provides crucial information for understanding the **medicinal value** of *Calendula officinalis* flower extracts.

Table No. 1: Phytochemical analysis of hydro-ethanolic flower extract of *Calendula Officinalis Linn*.

Sl. no.	Name of the test	Hydro-ethanolic extract of *Calendula Officinalis Linn*.
1	Test for Carbohydrates Molish test Fehling's test Benedict's test	+ + +
2	Test for Proteins: Biuret test Million's test	+ +
3	Test for Amino acids: Ninhydrin test Test for tyrosine	+ +
5	Test for Glycosides: Keller-killiani test Legal's test	+ + +

	Borntrager's test	
6	Test for Flavonoids: Shinoda test H_2SO_4 test NaOH test	 + + +
7	Test for Alkaloids: Mayer's test Wagner's test Tannic acid test	 + + +
8	Test for Phenolic compounds 5% $FeCl_3$ Solution	-
9	Test for Saponin: Foam test	+

Note: '+'=Present; '-' = Absent

CONCLUSION

The result shows the presence of phytochemicals such as carbohydrates, proteins, amino acids, glycosides, flavonoids, alkaloids, saponin and quinones in the flower extracts of *Calendula officinalis Linn.* These elements having potential application as a medicinal agent.

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REFERENCE

1. Or G, Rachel K, Denis V, Michelle C, RN, Josef H, Moti H.(2019). A systematic review of Calendula Officinalis extract for wound healing. *Wound Repair and Regeneration*, 1-14.
2. Darekar D. P, Hate M. S.(2021) Phytochemical Screening of Calendula Officinalis (Linn.) using gas-chromatography- mass spectroscopy with potential antibacterial activity. *Journal of Scientific Research*, 65(2): 6-12.
3. Muley BP, Khadabadi SS and Banarase NB. (2009). Phytochemical constituents and pharmacological activities of Calendula Officinalis Linn (Asteraceae): A Review. *Tropical Journal of Pharmaceutical Research*, 8(5): 455-465.
4. Ali Esmail Al-Snafi.(2015). The chemical constituents and pharmacological effects of Calendula Officinalis - a review. *Indian Journal of Pharmaceutical Science & Research*, 5(3): 172-185.

5. Monica B and Cristina Z. C. (2012). Evaluation of biologically active compounds from Calendula Officinalis flowers using spectrophotometry. *Chemistry Central Journal*, 6(35): 1-7.
6. Karthikeya P, C.J. Sanjay, Nagabhushana D, KR Renuka D, Harshitha N. (2022). A review of Calendula Officinalis- magic in science. *Journal of Clinical and Diagnostic Research*, 16(2): 23-27.
7. Garima D, Prashant D, Sonia T. (2022). Review of phytochemical constituents and pharmacological activities of the plant Calendula Officinalis Linn. *International Journal of Medical, Pharmaceutical, and Life Sciences*, 02(02): 216-228.
8. Bhargab D, Bedanta B, Anshul S, Abu Md Ashif Ikbal, Chayanika G, Santa S.(2021). Mechanism of action of wound healing activity of Calendula officinalis: A comprehensive review. *Pharmaceutical and Biosciences Journal*, 9(1): 28-44.
9. Neda B, Dariush M, Mohammad K, Fatemeh V, Yasaman M, Ali B, Mahmoud B, Mohammad T. K, Asieh K, Mahdi P and Ali A. M. (2013). Open access antioxidant capacity of Calendula Officinalis flowers extract and prevention of radiation induced oropharyngeal mucositis in patients with head and neck cancers: a randomized controlled clinical study. *DARU Journal of Pharmaceutical Science*, 21(18): 1-7.
10. Jyotisree G, Sruthi R, Biju C. R, Amita S. M. (2020). Calendula Officinalis and echinacae purpurae as antimicrobial agent. *Journal of Applied Pharmaceutical Research*, 8(2): 8-12.
11. Shalini S and Kavita K. (2021). An overview on Calendula Officinalis Linn.: (Pot Marigold). *Journal of Advanced Scientific Research*, 12(3): 13-18.
12. Rahul J, Mukund T, Sudarshan B, Nandini R, Prathamesh J, Samruddhi I. (2023). A review on Calendula Officinalis. *Research Journal of Pharmacognosy and Phytochemistry*, 15(1): 5-11.
13. Sagar J, Avantika B, Aakanksha C, Sharaddha Tand Vaishnavi G. (2024). Formulation and evaluation of microspongial gel of Calendula Officinalis for wound healing property. *International Journal Of Progressive Research In Engineering Management And Science*, 04(6): 2389-2405.
14. Hiba Ali H and Zahraa Abdul E A. (2020) Pharmacognostical and phytochemical study of Calendula Officinalis L leaves cultivated in Baghdad. *Applied Science and Technology*, 1-9.

15. Tinky S, Binjita P, Bishnu K. S, Gayatri M. K, Rojeena T, Nabin K.(2020). Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. *Tribhuvan University Journal*, 35(2): 1-11.
16. Dipak R. P, Narayan D. P, Dil B. S, Uday N. Y, Dharma P.K.(2017). Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of Pterocarpus marsupium Roxburgh. *Journal of Intercultural Ethnopharmacology*, 6(2): 170-176.