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PHYTOCHEMICAL SCREENING OF *CALENDULA OFFICINALIS* LINN LEAF EXTRACT BY TLC

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ABSTRACT

Identification of primary and secondary constituents has become the utmost important tool for the presence of active moiety. The phytochemical screening of petroleum ether, chloroform, methanol and water extracts of *Calendula officinalis* leaf done by TLC means. Petroleum ether extract showed the presence of fatty acids, chloroform extracts showed the presence of triterpens and sterols. Flavonoids, carbohydrates, amino acids and saponins were present in methanol extract and saponins, phenolic substances and tannins were present in the water extract of *Calendula officinalis.*

KEYWORDS: *Calendula officinalis*, Phytochemical screening, TLC

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INTRODUCTION

Calendula officinalis Linn (Asteraceae) known as Pot Marigold is an important medicinal plant used in our Traditional Systems of Medicine for treating various diseases like fever, and cancer. *Calendula officinalis*. Linn of Family Asteraceae has been widely used in homeopathic medicine for the treatment of many diseases[1]. It has been reported to possess many pharmacological activities, which include antioxidant [2], anti-inflammatory[3], antibacterial[4], antifungal[5] and antiviral[6]. It also possess cytotoxic as well as tumor reducing potential[7]. It is used as analgesic, anthelmintic, anti-bacterial, anti-emetic, anti-fungal, anti-inflammatory, anti-pyretic, antiseptic, anti-spasmodic, anti-viral, astringent, bitter, candidicide, cardiotonic, carminative, cholagogue, dermagenic, diaphoretic, diuretic, hemostatic, immunostimulant, lymphatic, uterotonic, and as vasodilator. Generally in cases of external it is used for treating skin inflammations, open wounds and laceration wounds with bleeding. It is also used for treating minor diseases like razor burns and wind burns. Internally it is used for mucous membrane inflammations, peptic and duodenal ulcers, spasms of the GI tract, duodenal and intestinal mucosa, dysmenorrhea (painful menstruation) especially in nervous or anemic women, splenic and hepatic inflammations. It is also used as a mouthwash after tooth extractions. The plant is rich in many pharmaceutical active ingredients like flavonoids, carotinoids, glycosides and sterols. It contains lupeol, quercetin, protocatechuic acid etc. many alkaloids and triterpinoids.

MATERIALS AND METHODS

Plant Material

Fresh leaves of the plant *Calendula officinalis* (Linn.) were obtained identified and authenticated from the voucher specimen SPTM/Cal 1.The collected leaves were dried in shade, crushed to coarse powder and used for further studies.

Preparation of Extract

The leaves of *Calendula officinalis* were collected, dried in the shade, powdered, weighed (1 kg) and they were subjected to continuous hot extraction in soxhlet apparatus. The extraction was carried out as per the polarity of the solvents with petroleum ether chloroform, methanol and finally with water for 36 hours. The solebt from each extraction was distilled off and the concentration was carried out on a water bat to appropriate consistency and then evaporated to dryness and used for the phytochemical analysis. The residue for each extract was petroleum ether (120 gm), chloroform (200 gm), methanol (300 gm) and finally water (100 gm)[8]. The extracts were subjected to preliminary qualitative tests to identify the various phytoconstituents present in leaves[9].

Amino acids

The amino acids can be detected as per the standard procedure depicted in Harbone 9. The plant material was mixed with 10 % isopropanol and ground using motor and pestle. The filtrate was centrifuged at 13000 rpm for 3 min and the supernatant was used for the detection of amino acid. Samples were spotted on TLC plate using n-butanol-acetic acid-water (12:3:5) as mobile phase. TLC plates were sprayed with 0.2% Ninhydrin in acetone, dried over at 105°C for 1-2 minutes. The presence of violet colour to pink colour indicates[10,11].

Essential oils

They were detected by using methelyne-di-chloride-chloroform –ethyl acetate-n-propanaol (47: 45: 2: 2.5) as mobile phase. TLC plates were sprayed with vanillin sulphuric acid reagent[12] and dried at 105°Cfor 2 minutes. Appearance of pink, brown colour spots shows the presence of essential oils.

Triterpenoids

The detection for triterpenoids was done by applying the samples on TLC plate impregnated with silver nitrate and they were developed in butanol-2M ammonium hydroxide (1:1) as mobile phase. The detection was done by spraying antimony trichloride. The purplish spot indicates the presence of triterpenoids.

Alkaloids

They can be detected by Dragendroff's reagent. The test sample was applied on precoated TLC plates developed in chloroform: Methanol (9:1) as mobile phase, dried sprayed with the reagent. Appearance of orange red spot at room temperature indicates the presence of alkaloid.

Saponin

Sample was applied on TLC plates developed in Chlorofrom-methanol-water (60:35:5) as mobile phase and dried. Plates were then sprayed with 1% vanillin 5% sulphuric acid reagent and dried at 110°C for few minutes glycosides appear as dark bluish to black spot.

Sterols

Generally steroids have a nucleus of cyclopentene perhydrophananthrene found in plant tissues called as phytosterols. In higher plants stigmasterol, sitosterol are the most common sterols found in abundant which are in free and in combined form. For the detection of steroids samples were applied on TLC plates developed in Chloroform- methanol (3:4) as mobile phase and were detected by spraying anisaldehyde. TLC plates were also developed in benzene- methanol (95:5) as another mobile phase and were detected by spraying 5% alcoholic sulphuric acid reagent. Bluish- green spots were observed[13].

Fatty acids

For the detection of fatty acids samples were applied on a pre coated TLC plates, aur dried and were developed in Hexane- ethyl acetate (95: 5) as mobile phase, sprayed with 5% alcoholic potassium permanganate solution. The appearance of dark brown spots indicates the presence of fatty acids.

RESULT AND DISCUSSION

The results of preliminary phytochemical screening of petroleum ether, chloroform, methanol and water extracts of *C.officinalis* leaves **(Table 1)** revealed that Petroleum ether extract showed the presence of fatty acids, chloroform extracts showed the presence of triterpens and sterols. Flavonoids, carbohydrates, amino acids and saponins were present in methanol extract and saponins, phenolic substances and tannins were present in the water extract of *Calendula officinalis.* From these results it can be depicted that this plant can be used for its various activities like analgesic, immunomodulatory effects, anti-inflammatory and for neurological behavior studies. Further isolation of the active constituents can be achieved from this data that which extract shows maximum phytochemical constituents and can be explored for single drug entity.

REFERENCES

1. Zitterl-Eglseer K. Sosa S. Jurenitsch J. Schubert-Zsilavecz M. Della Loggia R. Tubaro A. Bertoldi M. and Franz C. Anti-edematous activities of the main triterpendiol esters of marigold (*Calendula officinalis* L.). J Ethnopharmacol 1997; 57: 139–144.

2. Preethi KC, Kuttan G, Kuttan R. Antioxidant potential of *Calendula officinalis* Flowers *in vitro* and *in vivo*. Pharmaceutical Biol 2006; 44: (9: 691–697.

3. Della LR, Tubaro A. Sosa S, Becker H, Saar S, Issac O. The role of triterpenoids in the topical anti-inflammatory activity of *Calendula* flowers. Planta Medica 1994; 60: 516–520.

4. Dumenil G, Chemli R, Balausard G. Evaluation of antibacterial properties of *Calendula officinalis* flowers and mother homeopathic tinctures of *Calendula officinalis*. Ann Pharma Fran 1980; 38: 493–499.

5. Kasiram K, Sakharkar PR, Patil AT. Antifungal activity of *Calendula officinalis.* Indian. J Pharm Sci 2000; 6: 464–466.

6. Barbour EK, Sagherian V, Talhouk S, Talhouk R, Farran MT, Sleiman FT, Harakeh S. Evaluation of homeopathy in broiler chickens exposed to live viral vaccines and administered *Calendula officinalis* extract. Med Sci Monit 2004;10(8): 281–285.

7. Boucaud-Maitre Y, Algernon O, Raynaud J. Cytotoxic and antitumoral activity of *Calendula officinalis* extracts. Pharmazie 1988;43:220–221.

8. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, New Delhi; 2005.

9. Harbone JB. Phytochemical Methods-A Guide to Modern Techniques of Plant Analysis, Chapman and Hall London; 1998.
10. Willkonson PC, Neutrophill Adhesion Test: In Vank J K Ferreria S H, 1st ed. Vol.1, Handbook of experimental Pharmacology, Springer-Verlag, Berlin; 1978.
11. Puri A, Saxena R, Saxena RP, Saxena KC. Immunostimulant agents from *Andrographis paniculata*. J Nat Prod 1993; 56: 995-999.
12. Touchstone JC, Dobbins MF. Practical of Thin Layer Chromatography, John Wiley Sons,(NY): 1978.
13. Gritter RJ, Albers RJ., Non-destructive zone developing in thin-layer chromatography. J Chromatog1962; 9: 392.

Table 1: Preliminary phytochemical screening of *Calendula officinalis* leaf extract

S.No	Test	PE	CE	ME	WE
1	Amino Acid	-	-	+	-
2	Triterpenoids	-	+	-	-
3	Flavonoids	-	-	+	-
4	Saponins	-	-	-	+
5	Sterols	+	+	-	-
6	Fatty acids	+	-	-	-
7	Phenols	-	-	-	+
8	Tannins	-	-	-	+
9	Carbohydrates	-	-	+	-

PE-Petroleum ether extract, CE-Chloroform extract, ME-Methaloic Extract, WE-Water extract

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