Phytochemical analysis and antimicrobial effect of Marigold (Calendula officinalis) extract on infectious microorganisms

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Abstract

The use of antibiotics is helpful as a remedial treatment, when bacteria are the cause of the infection. Medicinal plants that are commonly used are good sources for safe and effective antibacterial agents. Plants have the capacity to produce a large number of organic phytochemicals as secondary metabolites for self-defence. Pharmacological studies have confirmed that Calendula officinalis exhibit a broad range of biological effects, such as, anti-inflammatory, immuno-modulatory and wound healing. Antimicrobial properties make it an effective drug for controlling infections**.**
KEYWORDS: Antimicrobial, phytochemicals, secondary metabolite.

INTRODUCTION

India has a very long history of using traditional alternative medicine -Ayurveda found in Hindu scriptures called Vedas, written over 6,000 years ago. Ayurvedic medicine predominantly uses plant and herbal drugs as the main course therapy for treating a number of diseases. It can be also defined as “Phyto-therapy” comprehended in a very sophisticated way. (Nikam et al., 2011). Herbal drugs have made their importance felt in the last few decades whose prevalence is continuously increasing in both developing and developed countries due to their natural origin and lesser side effects

In “The Annual Plant Review: Functions and Biotechnology of Plant Secondary Metabolites” it has been shown that, plants have developed effective defence strategies to protect themselves from Phyto-pathogenic microbes in their environment. Disease resistance in plants depends on the activation of coordinated and multi component defence mechanisms. One of the mechanisms is their ability to accumulate low-molecular-weight compounds (secondary metabolites) with high antimicrobial activities, such as alkaloids, coumarins, flavonoids, poly acetylenes, Quinones, tannins and terpenes (Hattenschwiler and Vitousek, 2000); Michael and Reichling 2010).

It is a short-lived aromatic herbaceous perennial, growing to 80 cm tall, with sparsely branched lax or erect stems. The leaves are oblong-lanceolate, 5–17 cm long, hairy on both sides, and with margins entire, occasionally waved, or weakly toothed. The inflorescences are yellow, comprising a thick flower head of 4–7 cm diameter, surrounded by two rows of hairy bracts. In the wild plant, they have a single ring of ray florets surrounding the central disc florets, generally of a more intense orange-yellow. The flowers appear all year long, where conditions are suitable. Calendula officinalis has a long history of usage of the folk systems because of its rich medicinal values (Fonseca et al. 2011).

Active components with its properties

The flowers of Calendula officinalis contain a large number of phytochemicals that include flavonolglycosides, triterpeneoligoglycosides, oleanane-type triterpene glycosides, saponins, and asesquiterpeneglucoside Moreover, calenduline and oleanolic acid glycosides, sterol glycosides, alpha-and beta-amyrin, taraxasterol, lapel, brain, faradiol, arnidiol, erythrodiol, Calenduladiol, coflodiolhave been found in various parts of the plants (Khan et al. 2011)

It was found that high contents of the flovonoid and phenolic phytochemicals in Calendula officinalis result in its antioxidant activity and support its high radical scavenging activity. Antibacterial properties of marigold flowers and Mother tinctures of Calendula
officinalis evaluated previously for its antimicrobial activity by Iqbal Hussain (2012). Pharmacological studies have confirmed that Calendula officinalis exhibit a broad range of biological effects, such as, anti-inflammatory (Amoian et al., 2010), immuno-modulatory, wound healing. Flavonoids present in Calendula officinalis also act as anti-oxidants, thereby protecting body cells from damage caused by a chemical process called oxidation. Calendula's high-molecular weight polysaccharides a stimulate immune system and has been researched for, as an immune booster (Fonseca et al., .2011).

METHODOLOGY

I-Extract of the flowers was prepared using cold-aqueous and alcoholic-Methanol extraction. HPTLC fingerprinting profile-As herbal extracts are contain many constituents, it is important to confirm the bioactive compound. It is critical to obtain reliable chromatographic fingerprint that represent pharmacologically active and chemically characteristic components present in the extract. The HPTLC fingerprinting profile is an important parameter of herbal drug standardization for proper identification of medicinal plants. The extracts along with the standard markers were subjected to HPTLC.

II-Sensitivity Testing-Pathological samples were collected from hospital. Samples were sub cultured on Nutrient agar, Blood agar, Mac-conkeys agar an identified using standard biochemical testing. The bacterial inoculums were adjusted to ($1.5x10^{8}$ CFU/ml), inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. Sterile paper discs, (6mm diameter) were placed on the surface of each MHA plate using a sterile pair of forceps, and impregnated with 20 µl of the herbal extract.

RESULTS

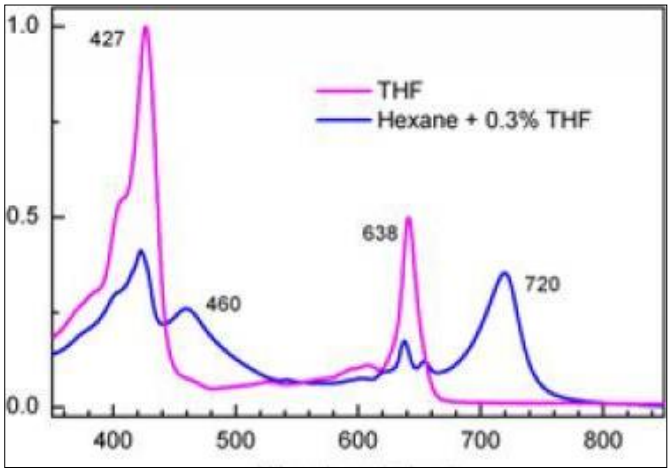
Table:1 .HPTLC OF THE EXTRACT:APPLICATION PARAMETERS

Instrument application	CAMAG Linomat 5	**Sequence**	
Spray gas	Inert gas	Syringe size	100 µl
sample solvent type	methanol	Number of tracks	6
Dosage speed	150 nl/s	Application position	8.0 m.m.
Pre dosage volume	0.2µl	Band length	8.0 m.m.
Application position	8.0 m.m.		

The alcoholic extracts showed distinct bands and on referring to standard R_f values, it could indicate the presence of Lutein (xanthophyl a naturally occurring carotenoid), lycopene. Comparing with standard references it can be correlated to the presence of Lutein
(Rf 0.817) as in the visible spectrum the extract showed a Maximum absorbance at 427nm Comparable to Standard Lutein-440nm

Table :2-DETECTION PARAMETERS- Lamp: D2/ Wave length: 254 nm

Tracks	**1**				**2**				**3**	**4**	**5**	**6**	
Applied sample	Alcoholic extract								Std Calendulin		Aqueous extract		
Applied volume	10µl				20µl				10µl	20µl	10µl	20µl	
R_f	0.31	0.44	0.55	0.81	0.32	0.45	0.55	0.60	- -	- -	0.63	0.63	0.8
Area of the peak	1327.4	1663.6	2732.4	270.1	2818.6	3517.4	5052.0	460.0	--	--	136.2	517.7	103.2



Spectrum scan:400-800nm
R_f: 0.38,
Absorption Max: 427 nm
Aqueous and Alcoholic extracts of Calendula

Table:3-Distribution of isolates

Culture	No.	Percentage
Klebsiella spp.	64	34.4
Pseudomonas spp.	41	22.04
Acinetobacter spp	13	6.98
E. coli	2	1.07
Proteus spp.	1	0.05
Streptococcus pyogenes	30	16.12
Candida albicans	17	9.13
Staphylococcus aureus	12	6.45
Micrococcus spp.	5	2.68
Corynebacterium diphtheriae	1	0.05
Total number of Isolates: n = 186		

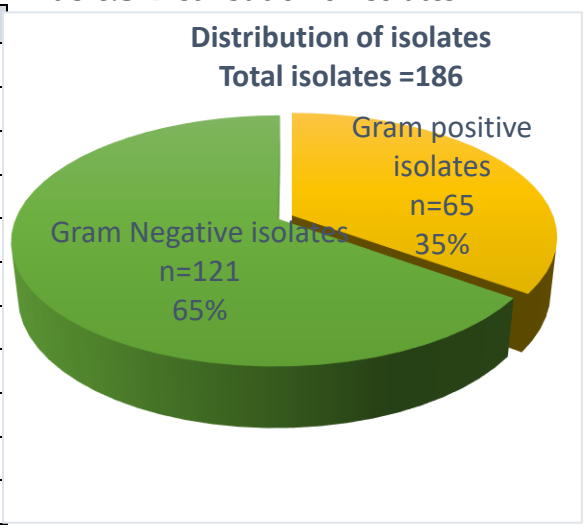
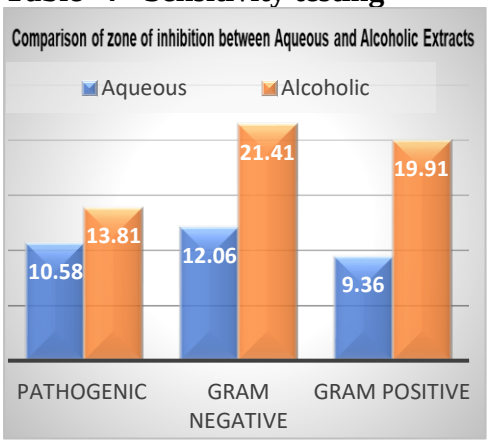


Table -4 - Sensitivity testing



Extracts	Mean zone size of inhibition		
	Patho-genic n=186	Gram positive n=65	Gram negative n=121
Aqueous	10.16	9.36	10.58
Alcoholic	15.92	19.91	13.81

CONCLUSION

Alcoholic extracts Calendula showed a mean zone of 13.81 and phenomenal zone of inhibition of 21.41 for gram negative isolates whereas Aqueous extracts of Calendula did not seem to be as effective as a mean inhibition of 10.78. The extraction was done using water and methanol. It was observed methanol enabled more efficient extraction of flavonoids, which in accordance to scientific literatures responsible for the active potential qualities in its curative action. This result was consistent with the result previously cited by Monica et al., (2012). Phytochemicals in particular phenolic acids and flavonoids, compounds extracted from Calendula officinalis are responsible through their antioxidant activity (Preethi et al., 2009).

DISCUSSION

There are reports that describe the use of a strong tincture of the flowers applied to wounds, reduce inflammation and suppuration, and to accelerate the healing of wounds. In the present study Calendula has exhibited potent antibacterial action on gram negative isolates. Calendula is one of the most valuable medicinal plants because a variety of **p**hytochemicals such as, terpenoids, flavonoids (particularly patulitrin and patuletin), coumarins, Quinones, carotenoids (lutein) and other compounds are present in this plant. The mechanisms underlying these possible effects are poorly understood. Other beneficial compounds identified in Calendula officinalis extracts by Muley et.al (2009) were proteins, amino acids, saturated hydrocarbons and vitamin-C. Though presence of calendulin could not be confirmed, flavonoids (particularly patulitrin and patuletin) or lutein could be responsible for effective bacterial inhibition in the present study.

Due to additional antioxidant, immune stimulating and other beneficial properties, Calendula can be used in aerosol applications, as a localized treatment in controlling infections.

REFRENCES:

Amoian, B., Moghadamnia, A., Mayandarani, M., Amoian, M., Mehrmanesh S (2010). The effect of Calendula extract on the plaque index and bleeding in gingivitis. Journal of medicinal plant research, vol-4(3):pp132-140.

Fonseca,Y.M., Catani,C.D., Vicentini,F.T., et al.,(2011) Efficacy of marigold extract-loaded formulations against UV induced oxidative stress. Journal of Pharmacological Science, vol-100:pp2182-2193.

Hättenschwiler, S., Vitousek, P.M., (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology and evolution, vol-15(6):pp 238-243

Iqbal, H., Jameel, A., Shumaila,N., Riaz Ullah, Shabir Ahmad, Sadar Jan , and Kamin Khan., (2012). Study on the medicinal plant Calandula officinalis. African Journal of Pharmacy and Pharmacology, vol-6(13):pp973-978.

Khan M.U., Ankur,R., Deepika,B., Shadan,A., (2011). Diverse Belongings of Calendula officinalis: An Overview. International Journal of Pharmaceutical Sciences and Drug Research,vol-3(3):pp173-177

Michael, W. and Reichling, J. (2010). Plant-microbe interactions and secondary metabolites with antibacterial, antifungal and antiviral properties.2nd edition. Annual Plant Reviews.vol-39: Functions and Biotechnology of Plant Secondary Metabolites.

Monica, H., Butnariu, C. Z., Coradini .(2012).CLE Open Access. Evaluation of biologically active compounds from Calendula officinalis flowers using spectrophotometry .Chemistry Central Journal, vol-6:pp35-38.

Muley, B.P, Khadabadi, S.S., Banarase, N.B.,(2009). Phytochemical Constituents and Pharmacological Activities of Calendula officinalis .Tropical Journal of pharmaceutical Research, vol-8:pp455-465.

Nikam, P, H., Joseph, K., Aruna, J., Kadam, V. (2011). Future Trends in Standardization of Herbal Drugs. Journal of Applied Pharmaceutical Science, vol-2 (6):pp38-44.

Preethi, K.C., Kuttan, R. (2009). Wound healing activity of flower extract of Calendula officinalis. Journal of Basic Clinical Physiology and Pharmacology, vol-20: pp73-79.