WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 15, 488-496. Research Article ISSN 2277– 7105

HPLC ANALYSIS OF CALENDULA OFFICINALIS AND AZADIRACHTA INDICA

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Article Received on
03 June 2018,

Revised on 23 June 2018,
Accepted on 14 July 2018,

DOI: 10.20959/wjpr201815-12903

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ABSTRACT

Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents. Almost every part of the tree is bitter and finds application in indigenous medicine. Natural drugs have been a part of the evolution of human, healthcare for thousands of years. Nowadays nearly 88% of the global populations turn to plant derived medicines as their first line of defence for maintaining health and compacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered. In the Current study, isolation of active components through Thin Layer Chromatography (TLC) and High performance liquid chromatography (HPLC).

KEYWORDS: Thin Layer Chromatography (TLC) and High performance liquid chromatography (HPLC).

INTRODUCTION

Natural drugs have been a part of the evolution of human, healthcare for thousands of years. Nowadays nearly 88% of the global populations turn to plant derived medicines as their first line of defence for maintaining health and compacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains. Presently 80 percent of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases.

Calendula officinalis has a long history of usage by the folk systems because of its rich medicinal values that have been reported to possess potent anti-inflammatory, antitumour, antioxidant, antibacterial, anti-HIV, anti-ulcer, antigenotoxic, chemoprotective and antiseptic properties.[1] Moreover, a large number of phytochemicals have been found in various parts of the plants that include calenduline and oleanolicacid glycosides, sterol glycosides, alpha-and beta-amyrin, taraxasterol, lupeol,brein, faradiol, arnidiol, erythrodiol, calenduladiol, coflodiol and manilladiol.[2-3]

Antibacterial properties of marigold flowers and mother homeopathic tinctures of C. officinalis been evaluated previously.[4] The sap of different organs of Calendula sp. has been studied for antimicrobial activity by.[5] The present study will however explore new frontiers and will open new doors for the herbal industries, local practioners and for other users and a scientific data basis for the traditional claims of this ethnic medicinal plant.

Various tests and methods have been developed and adapted to specifically assess the presence and activity of antioxidants in foodstuffs, nutraceuticals, dietary supplements, and biological fluids.[6-7]

Neem is a moderate sized to large, usually evergreen tree, with a fairly dense crown and glabrous leaves divided into leaflets. The bark is fairly thick, furrowed longitudinally or obliquely and is dark grey outside and reddish brown inside. The tree in Maiduguri flower

throughout the year but fruits during the cold harmattan season which corresponds with the winter of temperate climates. The fruits are yellowish green when ripe and have a sweetish pulp containing one seed. In Northern Nigeria, the neem plant is used in traditional circles for the treatment of general body pain after child delivery, pyorrhea, and intestinal worms.[8] Based on this traditional and other uses of Azadirachta indica, this study was conducted to as certain its potentially pharmacologically active components.

NEEM TREE

Classification

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	A. indica
Scientific Name	*Azadirachta indica*
Found In	Ranthambore National Park, Bandhavgarh national Park, Mrugavani Naional Park, Bannerghata National Park, Sariska Wildlife sanctuary and Guindy National Park.

Neem oil extracted from its seeds is used in medicines, pest control and cosmetics etc. Its leaves are used in the treat Chickenpox. According to the Hindus, it is believed that the Goddess of the chickenpox, Sithala lives in the Neem tree. Neem tea is usually taken to reduce the headache and fever.

Marigold Plant

Classification

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asrerales
Family	Calenduleae
Genus	Calendula
Scientific Name	*Calendula officinalis*
Found In	Valley of Flowers, Ranthambore National Park

They are even used to decorate the religious places. The leaves of its flowers are used as salads. Yellow dye has also been extracted from the flower, by boiling. The burning herb repels insects and flies. Pigments in the Marigold are sometimes extracted and used as the food colouring for humans and livestock.

MATERIALS AND METHOD

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The fresh leaves of *Calendula Officinalis and Azadirachta Indica* formulation were collected from in and around areas of Tiruchirappalli, Tamilnadu. The plant species was identified and authenticated at Rapinat Herbarium, St. Joseph College, Tiruchirappalli, Tamilnadu.

PREPARATION OF LEAF POWDER

The leaves of *Calendula Officinalis and AzadirachtaIndica formulation* was washed with sterile distilled water thrice, cut into small pieces and shade dried at room temperature for

two weeks and made into a coarse powder using mechanical blender and stored in an airtight container.

UV -VIS Spectral Analysis

The UV-VIS spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and 800 nm ranges.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography is an important tool in the separation, identification and estimation of different components. Here the principles of separation are adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phases, its preparation and use with different solvents can be achieved on the basis of partition and adsorption. The plant extracts showed good resolution in solvent system by trial and error method Generally Toluene: Acetone, Benzene: Ethyl acetate, n-Hexane: Acetone etc solvents are used.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is also known as High- Pressure Liquid Chromatography. This separates compounds on the basis of their interaction with solid particles of a tightly packed column and the solvent of the mobile phase. High pressures of up to 400 bars are required to elute the analyte through the column before they pass through detector. HPLC is useful for compounds that cannot be vapourised or that decompose under high temperatures. HPLC provides both quantitative and qualitative analysis in a single operation.

RESULT AND DISCUSSION

Table 2: Thin Layer Cromotography Of *Calendula Officinalis And Azadirachta Indica Formulation.*

S. No.	Extracts	Spot	R_f Value
1	Crude water	Brown Spot	0.50
2	Crude alcohol	Green Spot	0.83

THIN LAYER CHROMATOGRAPHY

TLC analysis also suggests the presence of different kinds of phytochemicals in *Calendula Officinalis and Azadirachta Indica formulation*. TLC profiling of all extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various

phytochemicals gives different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity. Thin layer chromatography was performed on Calendula Officinalis and Azadirachta Indica formulation using alcohol. TLC of Calendula Officinalis and Azadirachta Indica formulation of alcohol extract reports four spots for various phytochemicals. The reported spots are separated with enough space and having various $R_f$values showing the presence of phytochemicals in alcohol extract. Four bands found in this method and its R_f values were 5.7, 6.8, 5.9 and 8.0. This values indicate the presence of alkaloids (Fig 5 and Plate 3).

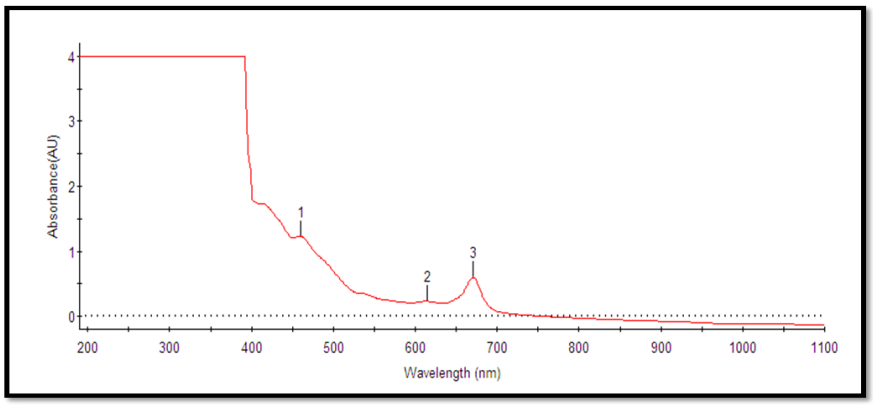


Fig. 1: uv-vis-Spectroscopy Ofcalendula Officinalis and Azadirachta Indica Formulation.

Table 3: Uv-Vis-Spectroscopy Of *Calendula Officinalis And Azadirachta Indica Formulation.*

S.NO	Wave Length	Absorbance
1	613.65	0.2301
2	670.75	0.5991

UV-VIS Analysis

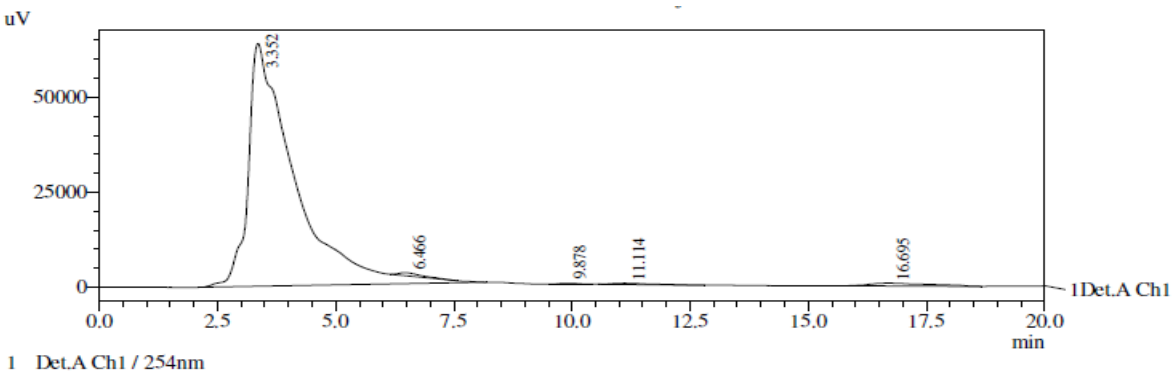
The qualitative UV-VIS profile of ethanolic extract of *Calendula Officinalis and Azadirachta Indica formulation*was taken at the wavelength of 300 nm to 800nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 613 and 457nm with the absorption 0.2301, and1.5088 respectively. Figure 1 shows the absorption spectrum of *Calendula Officinalis and Azadirachta Indica formulation* extract and these are almost transparent in the wavelength region of 200-1100 nm. Absorption bands observed pertaining to *Calendula Officinalis and Azadirachta Indica formulation* are displayed in Table 3 and fig 6. In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400

nm is a clear indication of the presence of unsaturated groups and hetero atoms such as S, N, O. The spectrum for *Calendula Officinalis and Azadirachta Indica formulation* shows two peaks at positions 613 nm, and 457 nm. This confirms the presence of organic chromophores within the *Calendula Officinalis and Azadirachta Indica formulation*.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC profile of *Calendula Officinalis and AzadirachtaIndica formulation*reveled 3 major peaks as item 1, item 2, item 3 with retention time 3.352 min, 6.466 min, 9.878 min respectively. The peak number 3 with retention time 9.878 of *Calendula Officinalis and AzadirachtaIndica formulation*.is considered as major compound of phenol, As comparing to the standard.

According to HPLC fingerprinting is the best way for chemical characterization, and therefore this study also established HPLC fingerprint for the active phenlic acids that can act as antioxidant, antifungal, antibacterial and anti-inflammatory. HPLC analysis revealed presence of a variety of phenolic compounds in extracts of *Calendula Officinalis and Azadirachta Indica formulation* which might have been responsible for their effective therapeutic potential.[9] However, Phenolic compounds can be defined as a large series of chemical constituents possessing at least one aromatic ring bearing hydroxyl and other sub constituents, including their functional derivatives. Based upon the HPLC fingerprints, it can be concluded that this analytical technique is a convenient method to identify the presence of numerous constituents present in the ethanolic extract.



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.352	4380156	63881	97.364	96.844
2	6.466	28082	753	0.624	1.142
3	9.878	6356	234	0.141	0.354
4	11.114	17890	338	0.398	0.513
5	16.695	66239	757	1.472	1.147
Total		4498723	65962	100.000	100.000

Fig. 2: High Performance Liquid Chromatography.

Azadirachtin extracts from the seeds, leaves and bark of the Neem tree has been reported to have strong biological activities against insect pests, but with very low toxicity to mammals and the environment, generally.[10] Registered Neem insecticide formulations Neemros® and Neemroc EC® have also been found to be effective against insect pests of vegetables but safe to their natural enemies.[11]

CONCLUSION

Based upon the HPLC fingerprints, it can be concluded that this analytical technique is a convenient method to identify the presence of numerous constituents present in the ethanolic extract of plant formulation.

ACKNOWLEDGEMENT

The authors are grateful thank to the Secretary, Trust members and principal of Rabiammal ahamed Maideen womens college for Women, Tiruvarur -610001, Tamilnadu, India for providing necessary laboratory facilities to complete this manuscript.

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