Qualitative and Quantitative Analysis of Stigmasterol from Iraqi Calendula officinalis

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KEYWORDS

Calendula, Stigmasterol, isolation

ABSTRACT

Calendula officinalis L. (Asteraceae) plant origin from Mediterranean countries, and in addition to the beauty of its flowers they also have important medicinal properties in the healing of many illnesses particularly skin issues. C. officinalis in the world was reported and assessed as a medicinal plant, like British pharmacopoeia (BP), European pharmacopoeia (EU), WHO and PDR. Calendula officinalis Linn. Contains 0.4% of flavonoids as minimum, calculated as Hyperoside of dried herbal substance according to British pharmacopeia (2). The Chemical Constituents are: Triterpene saponins, Triterpene (Stigmasterol), Flavonoids: isorhamnetin glycosides and quercetin glycosides, Coumarins: umbelliferon, scopoletin and aesculetin, Carotenoids: mainly zeaxanthin and Lutein (5). Stigmasterol is an essential ingredient that has been isolated from plants. Stigmasterol considered a progesterone precursor and serves as an intermediate in corticoids, androgens estrogens biosynthesis (10) and vitamin D3 synthesis (3). Stigmasterol is a triterpenes C30-derive phytosterol, naturally happening C28 and C29 carbon steroid alcohols that is similar to cholesterol in structure as well as biological activity (4).in this study Stigmasterol was identified by TLC, Mass analysis. Structure elucidation was performed using, FTIR and Mass analysis. The quantity of Stigmasterol was measured in the Iraqi plant.

1. Introduction

Calendula officinalis, known as pot marigold. The public name "marigold" mentions Virgin Marry. Considered the most common cultivated in use member of this genus is pot marigold (Calendula officinalis) [(9)]. *Calendula officinalis* Linn. Contains 0.4% of flavonoids as minimum, calculated as Hyperoside of dried herbal substance according to British pharmacopeia [(2)]. The Chemical Constituents are: Triterpene saponins, Triterpene (Stigmasterol)Flavonoids: isorhamnetin glycosides and quercetin glycosides, Coumarins: umbelliferon, scopoletin and aesculetin, Carotenoids: mainly zeaxanthin and Lutein [(2)]. *Calendula officinalis* Linn, in tradition was useful in treating internal organs, dysmenorrhea and gastrointestinal ulcer also was used as diuretic. Flowers were used as anti-inflammatory for oral and pharyngeal mucosa, also treat burns and wounds [(12)]. *C.officinalis* considered as detoxifying, cleansing herb and in the treatment of chronic infections [(1)]. Dried flowers were beneficial as anti-tumor and antipyretic effects [(7)]. Infusion preparation of flowers was used as antiseptic and antifungal in skin injuries and conjunctivitis [(8)]. C. officinalis tea was beneficial as gargles, eye washes and skin diseases or topical inflammation [(6)]. *C. officinalis* tinctures used in homoeopathy as treatment for insomnia and mental tension [(11)]. Stigmasterol considered a progesterone precursor and serves as an intermediate in corticoids, androgens estrogens biosynthesis [(10)] and vitamin D^3 synthesis [(3)]. It is triterpenes C30-derive phytosterol, naturally happening C28 and C29 carbon steroid alcohols that is similar to cholesterol in structure as well as biological activity [(4)]. Stigmasterol structure is illustrated in figure (1)

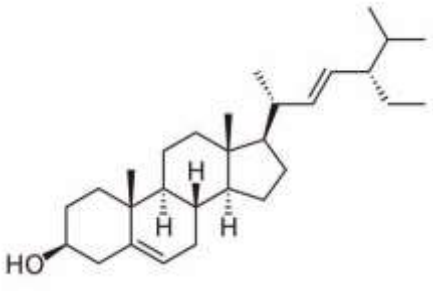


Figure 1: Stigmasterol structure.

2. Methodology

Analytical TLC, Preparative thin layer chromatography HPTLC (CAMAG), FTIR spectroscopy and Mass analysis. Standard Stigmasterol was obtained from Sigma Aldrich Company.

Plant Material

Flowers of *Calendula officinalis* collection from medicinal plants gardens at Mustansiriyah University/ pharmacy college. Plant was identified by the national herbarium in Abu-Graib, Baghdad. *C.officinalis* flowers collection during *February* & *March* then dried at room temperature in the shade, then grinded as powder and weighed. Extraction and isolation of Stigmasterol from dried plant material (100 gram) were extracted with hexane by soxhlet apparatus (700 ml), extract filtration and solvent evaporation under low pressure by rotary evaporator. Extract hexane was analyzed to detect the presence of stigmasterol using thin layer chromatography with spraying reagent and confirmed by: GC-MS analysis

Detection of Stigmasterol by Analytical TLC: Concentrated hexane extract was applied on an analytical thin later chromatography plate pre-coated with silica gel GF 254, then development was performed in three mobile phases: • S5: acetone: chloroform (1:9) [12]. • S6: ethyl acetate: hexane (2:7) • S7: chloroform: ethyl acetate : Toluene (4:1:5). The Rf value were compared with Rf value of standard stigma sterol. The spots were detected under UV 365 nm.

Preparative TLC: Concentrated hexane extract of 100 grams dried flowers was subjected to preparative TLC that preformed on pre-coated plates, then developed in mobile phase which was chloroform: acetone (90:10) [11], the detection of the spots was done by spraying plate side with reagent of vanillin-sulphuric acid (on plate side) first solution I then sprayed immediately with solution II, finally heating the plate for 5-10minutes at 110^{o}C with monitoring [11].

3. Results and discussion

Extract of hexane was analyzed to detect Stigmasterol presence by (TLC) as shown in Figure (2) with spray reagent (vanillin-sulphuric acid) [14], mobile phases were used and values of R_f are shown in Table (1).

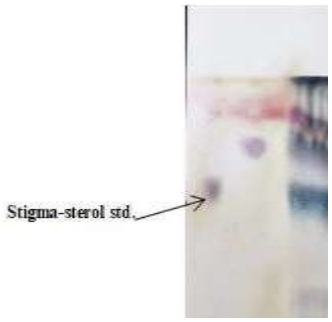


Figure (2): TLC plate reveal hexane extract against standard (Stigmasterol) S6: hexane: ethyl acetate (7:2)

Table (1): Rf of Stigmasterol reference standard and compound NA2 in the hexane extract of flowers using different solvent system in TLC.

Mobile phases	**Value of R_f of NA2 in extract**	**Value of R_f of Stigmasterol std**
S5: acetone: chloroform (1:9) [135]	0.7	0.71
S6: ethyl acetate: hexane (2:7)	0.48	0.485
. S7: chloroform: ethyl acetate : Toluene (4:1:5)	0.38	0.40

Structure elucidation of Stigmasterol:

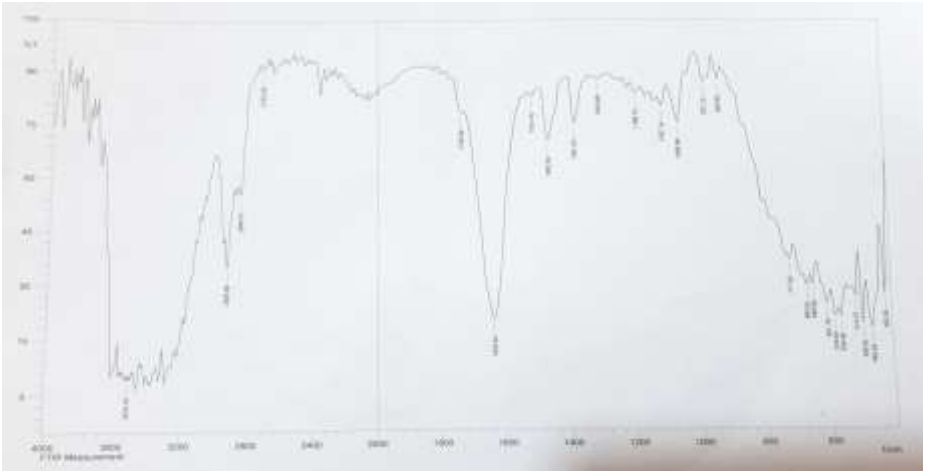
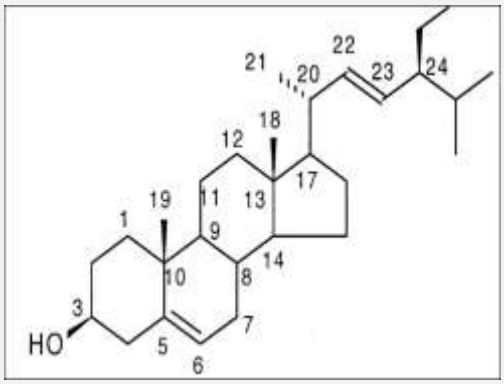
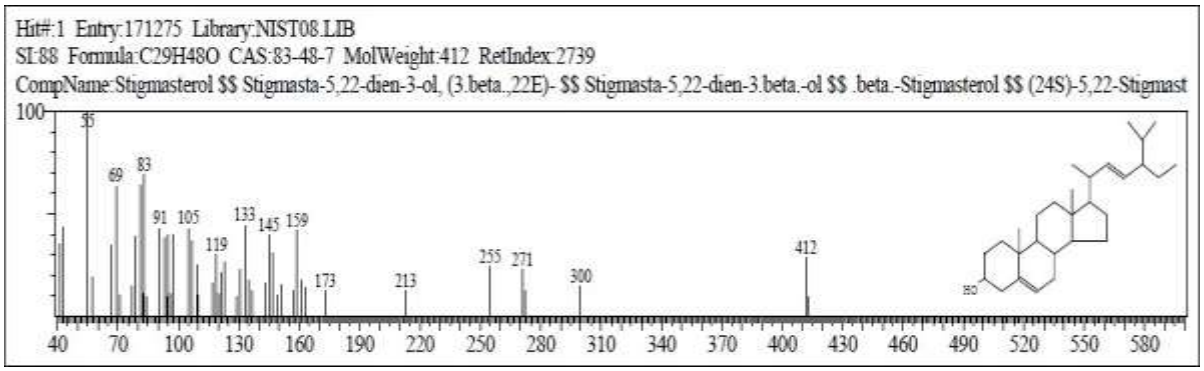


Figure 3: FTIR spectroscopy for isolated Stigmasterol

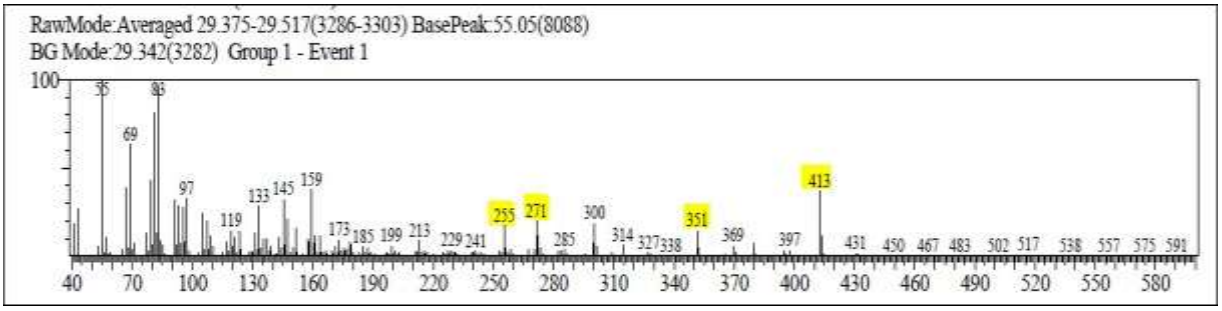
Table (2):IR bands with their interpretations of isolated Stigmasterol

Compound	**Bands (cm⁻1)**	**Interpretation**
	3518.16	broad Stretching vibration of OH
	2935 & 2858	Stretching vibration of CH aliphatic (asymmetric and symmetric)
	1635	Stretching vibration of C=C
	1462	Bending vibration of aliphatic.
	1056	Bending vibration of C-O of 2º alcohol

Mass analysis of isolated Stigmasterol



A



B

Figure (4): Mass spectrum of Stigmasterol. A: standard, B: isolated Stigmasterol

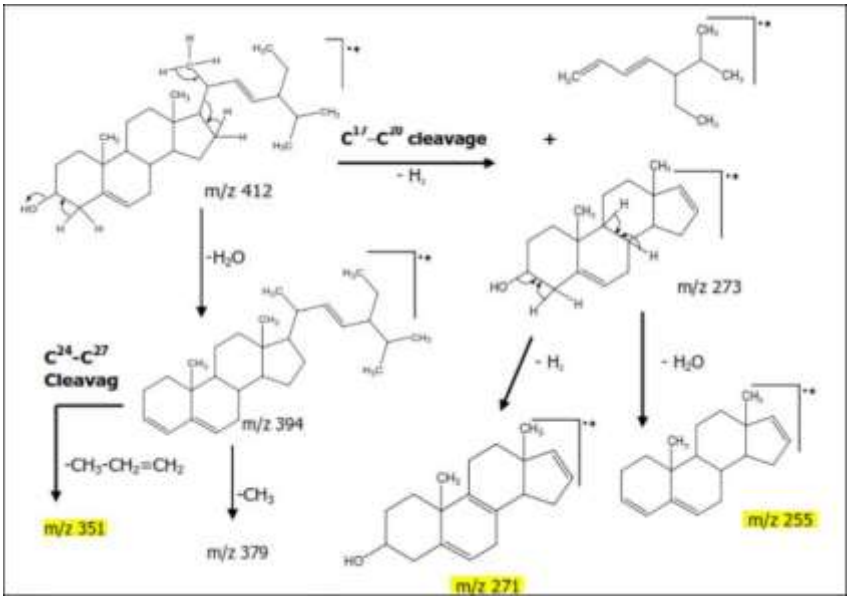


Figure (5): Fragmentation pattern of isolated Stigmasterol

Isolated Stigmasterol which was confirmed by TLC, IR, and MS analysis.

0.0127 g of Stigmasterol could be isolated from 50 g dried flowers, thus its concentration in plant = 0.0254 % w/w.

4. Conclusion and future scope

C. officinalis L is one of the important medicinal plants cultivated in Iraq.

Stigmasterol isolated from C. officinalis L is an important active constituent since its considered a progesterone precursor and serves as an intermediate in corticoids, androgens estrogens biosynthesis

(10) and vitamin D3 synthesis (3).

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