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# Isolation and identification of Phytosterols from *Plumbago capensis* L.

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## A B S T R A C T

Natural products are the most significant agents of life saving drugs globally. Phytochemicals have acted as drugs for decades and 50% medicines in these days are derival from bioactive compounds. Flora is regarded as molecular factory synthesizing enormous diversity of by-products called as "*Bioactive compounds"* i.e., plants are the richest source of many compounds like flavonoids, polyphenols, polysaccharides, alkaloids, Phytosterols, vitamins, tannins and lignin etc., represented as phytochemical. Phytosterols from *Plumbago capensis* L. was identified and quantified in vivo. Phytosterols were identified using chromatographic and spectral studies. ß-sitosterol, stigmasterol, campesterol and lanosterol were identified by IR and GC-MS. Total amount of phytosetrol were found in *Plumbago capensis* L. (0.62 mg/gdw in stem and 3.10 mg/gdw in leaves). GC-MS profiling showed 26 compounds. (Z) 6, (Z) 9 Pentadecadien 1ol (area of % 39.63) was found in highest amount.

***Key words*:** *Plumbago capensis*, Phytosterols, IR, TLC, GC-MS

The *Plumbago capensis L.*is a scrub and thicket plant, favouring the beautiful valley Bushveld of South Africa. It is commonly found in the Southern Cape and Eastern Cape right through to the balmy subtropical province of KwaZulu-Natal [1-2]. *Plumbago capensis* L. an evergreen shrub, often grown as a climber, ascending rapidly to 6m (20ft) tall by 3m (10ft) wide in nature, though much smaller when cultivated as a house plant [3]. Aerial parts of *Plumbago capensis* L. extracted in the following solvents: acetone, chloroform, petroleum ether, ethanol and ethyl acetate showed positive results when tested for steroids, carbohydrates, phenolics, tannins, saponins, flavonoids and terpenoids, however the aqueous extract only found positive for the presence of tannins [4]. Mostly secondary metabolites produced by some medicinal plants are involved in plant defense responses and facilitate plant adaptation to their environment by enhancing their general fitness and well-being [5]. Plant sterols often called as "Phytosterols" belong to the group of triterpenes, and they may contain an extra methyl or ethyl group at C-24 and a double bond at C-22. Sterols found in plants are known as phytosterols and over 250 phytosterols and their related compounds have been identified [6]. Plant sterols are present as mixtures in plants. Oehrl *et al.* [7] studied the oxidative stability of plant sterols in canola, coconut, peanut and soybean oils and found that sterol losses were greater in canola and soybean oils than in more saturated oils.

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## MATERIALS AND METHODS

*(i) Collection, identification and extraction of plant material*

The experimental plant sample was collected from Narayan vihar at Jaipur. The plant materials were taxonomically identified and authenticated by Department of Botany university of Rajasthan (RUBL no. 211571) Jaipur. Stem and leaves of selected plant were cleaned, shade dried and grinded to powdered and kept for further use. Dried and powdered plant material was defatted in petroleum ether (60-80°C) for 24 h on a water bath. Defatted material was air dried and hydrolyzed in 30% HCl (v/v) for 4 h. Each hydrolyzed sample was washed with distilled water till pH 7 was achieved and was dried later. The dried preparation was again extracted with benzene for 24 h. The extract was filtered and dried *in vacuo*. The crude extract was dissolved in benzene before chromatographic examination [8].

*(ii) Qualitative analysis*

*Thin layer chromatography (TLC)*- Glass plates coated with silica gels G were used. Each of the extract was co-chromatographed separately with authentic sterols as marker. These plates were developed in an airtight chromatographic chamber, saturated with solvent mixture (Hexane: Acetone:: 8:2; [9]. Other solvents such as benzene and ethyl acetate (85:15; Heble [10] benzene: ethyl acetate (3:1, [11] was also used but hexane: acetone (8:2) gave better separation. These plates were air dried and visualized

under UV light and fluorescent spots corresponding to that of standards marker were marked. These developed plates were sprayed with 50% sulphuric acid [12] and anisaldehyde reagent, separately and heated at 110°C for 10 min.

*Preparative thin layer chromatography*- PTLC was performed using silica gel G coated plates (0.4-0.5mμ) along with the reference markers. These plates were developed in hexane: acetone (8:2), air dried and examined under UV light. Each spot coinciding with that of standard marker was marked, scraped from 50 plates, and eluted with chloroform. The eluted reactions were subjected to crystallization, separately and their melting point, mixed melting point were determined. The isolated compounds were also subjected to UV and IR spectral studies.

*(iii) Identification*

Melting point and IR spectra of each of the isolated compounds was taken and a comparison of the TLC colour reaction was made, which was found to be in accordance with those of studied authentic compounds.

*(iv) Quantitative analysis*

*Thin layer chromatography (TLC)*- In plant parts along the levels of β-sitosterol, stigmasterol, campesterol and lanosterol were estimated by spectrophotometer following the method of [13] which included the computation of standard curves.

Stock solutions (1mgL$^{-1}$) of β-sitosterol, stigmasterol, lanosterol and campesterol were separately prepared in chloroform. Aliquots containing different concentrations (0.01 to 0.09 mgL$^{-1}$) were separately spotted on TLC plates and the developed air-dried chromatograms were visualized under UV light. The fluorescent spots were marked and collected with the absorbent in separate test tubes. Chloroform (5mL) was added in each test tube, shaken vigorously, centrifuged and supernatants were collected separately which were later evaporated to dryness. To each of this 3mL of glacial acetic acid was added. Shaken for 1 minutes at room temperature and tubes were placed in ice chest. To these test tubes 2mL of freshly prepared chromogenic reagent (0.5mL of 0.5% anhydrous $FeCl_3$ in glacial acetic acid +100mL of 3.6M $H_2SO_4$ was added drop wise at 0°C) and mixed thoroughly [14]. Each of the reaction mixture was incubated at 40°C for 30 min and their optical densities were measured using a spectrophotometer set at 540 nm against blank (3mL of glacial acetic acid +2mL of chromogenic reagent). In each case three replicates were run and mean value for each concentration was plotted against the respective concentrations to compute regression curves, which followed Beer's law.

Similarly, the extracts of all the isolated compounds dissolved in benzene were spotted on TLC 2 plates along with reference markers and processed as above. The coinciding spots with the reference compounds in these extracts were marked, scraped, eluted dried, taken up in 5mL of chloroform, and processed further, like the authentic samples. The concentration of β-sitosterol, stigmasterol, campesterol and lanosterol was calculated (mg/gdw) by referring the experimental samples with the respective regression curve, four such replicates were run and their mean value was calculated.

*Gas chromatography and mass spectroscopy (GC-MS)*- The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100°C (3 min) to 280°C at 1 to 40°C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 mL/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

## RESULTS AND DISCUSSION

In the present investigation, four of the phytosterols viz., β-sitosterol, stigmasterol, campestrol and lanosterol were extracted and identified from *Plumbago capensis* L. Sterols were spotted which were common in plant parts on thin layer chromatography. When the TLC plates were visualized under UV lamp four of the spots gave characteristic fluorescence and their Rf values were comparable to their respective standard compounds. The $R_f$ values of the spots matched with authentic standards and were identified as β-sitosterol, stigmasterol, campestrol and lanosterol. Among the various solvent systems tested best results were obtained in the solvent system Hexane: Acetone (8:2) with $R_f$ values viz., β-sitosterol, 0.89; stigmasterol, 0.83; campesterol, 0.29 and lanosterol, 0.23. The characteristic colours were also developed when TLC plates were sprayed with anisaldehyde reagent (β-sitosterol – Pink, Stigmasterol – Purple, campestrol – Blue and Lanosterol - Pink) and with 50% sulphuric acid (β-sitosterol-purple brown, Stigmasterol - Gray, Campesterol – Gray, Lanosterol – Pink brown) corresponding to their authentic samples.

Table 1 Chromatographic behavior and physicochemical characteristics of isolated phytosterols

| Isolated compounds | $R_f$ value | | | Color after spray | | M.P. (°C) | IR Spectral Peaks (rept.) ν (KBr) cm$^{-1}$ |
|---|---|---|---|---|---|---|---|
| | $S_1$ | $S_2$ | $S_3$ | $R_1$ | $R_2$ | | |
| β-sitosterol | 0.89 | 0.90 | 0.71 | PU-BN | PK | 136-137 | 3350 (O-H), 2830, 1665 (C=C), 1470, 1300, 1052 (C-O) and 880 |
| Stigmasterol | 0.83 | 0.64 | 0.65 | GY | PU | 167-169 | 3400 (O-H). 2950, 1750, 1640 (C=O), 1035 (C-O), 991, 957, 935, 810 and 715 |
| Campesterol | 0.29 | 0.23 | 0.21 | GY | BL | 157-158 | 3400 (O-H), 2950, 2850, 1640 (C=O), 1470, 1380, 1035, 880 and 820 |
| Lanosterol | 0.23 | 0.96 | 0.74 | PK-BN | PK | 143-144 | 3450 (O-H), 2890, 1640(C=O), 1385, 1360, 1310, 1290, 1270, 1110 and 880 |

Abbreviations: $S_1$- Hexane: acetone (8:2), $S_2$- Benzene: acetone (2:1), $S_3$- Benzene: ethyl acetate (3:2), $R_1$- 50% $H_2SO_4$, $R_2$- Anisaldehyde reagent, BN- Brown, PK- Pink, PU- Purple, BL- Blue, GY- Gray

The isolated sterols were also identified and characterized with their mp, which also corresponded with those of their respective standards separately (β-sitosterol 136-137°C, Stigmasterol 167-169°C, Campestrol 157-158°C and Lanosterol 143-144°C). The characteristic peaks of IR spectra of isolates (β-sitosterol, and stigmasterol) were also found to be super impossible with the IR spectra of reference compounds.

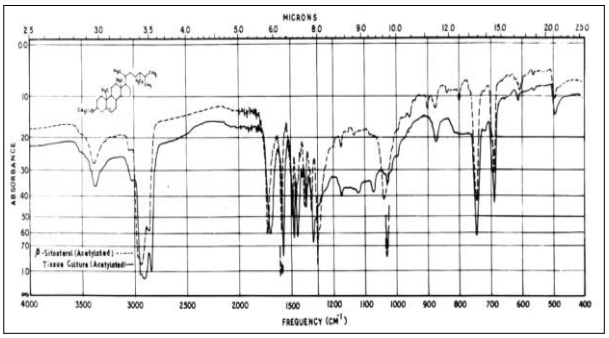


Fig 1 Infra-red spectra of isolated and standard β-sitosterol

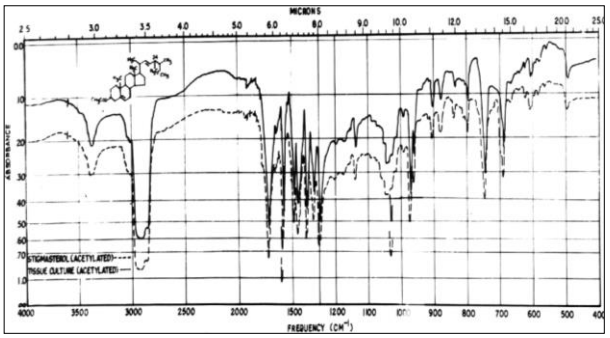


Fig 2 Infra-red spectra of isolated and standard stigmasterol

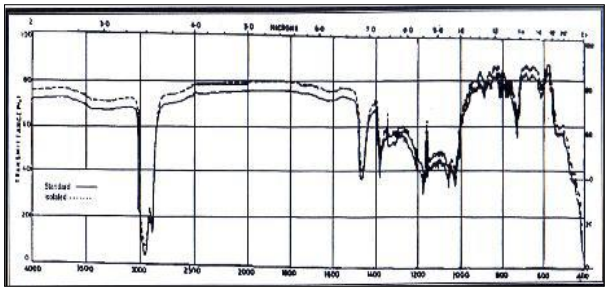


Fig 3 Infra-red spectra of isolated and standard campestrol

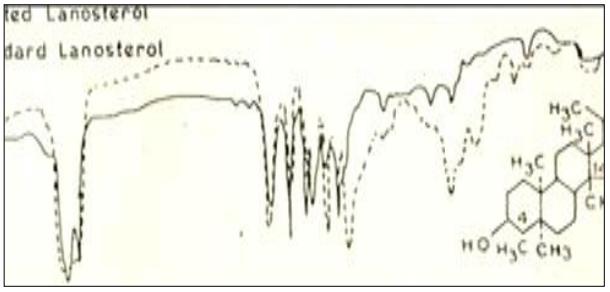



Fig 4 Infra-red spectra of isolated and standard lanosterol

Table 2 Yield of phytosterols isolated (mg/gdw) from various plant parts of *Plambago capensis*

| Plant parts | β-sitosterol | Stigmasterol | Campestrol | Lanosterol | Total (mg/gdw) |
|---|---|---|---|---|---|
| Stem | 0.32 | 0.15 | 0.08 | 0.07 | 0.62 |
| Leaves | 1.42 | 1.20 | 0.23 | 0.25 | 3.10 |

*Quantitative analysis*

Maximum phytosterol content was found in leaves (3.10 mg/gdw) of *Plumbago capensis* L. It was then also observed that 0.62 mg/gdw in stem. β-sitosterol of leaves (1.42 mg/gdw) than the stem (0.32 mg/gdw). If individually counted, then the stigmasterol of leaves (1.20 mg/gdw) was more than that of stem (0.15 mg/gdw). Then the campesterol of leaves (0.23 mg/gdw) was followed by lanosterol of leaves (0.25 mg/gdw).

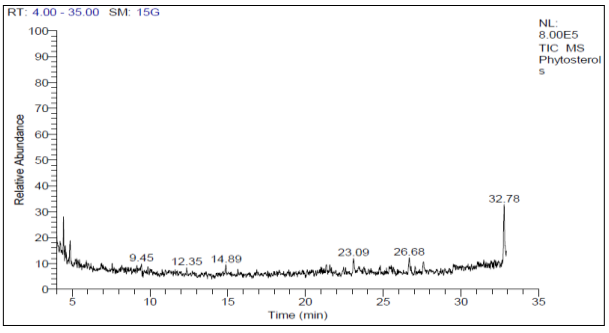
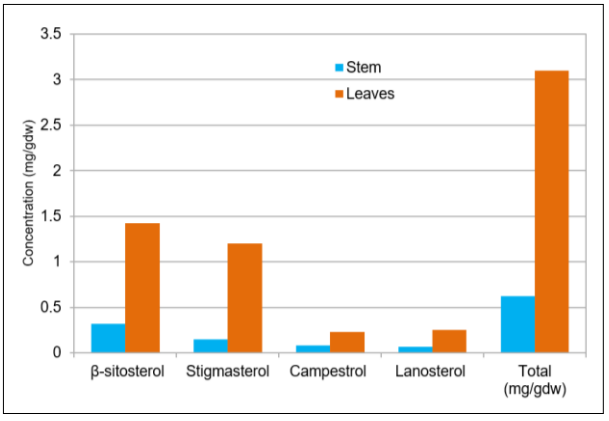



Fig 5 Showing GC-MS of phytosterol isolated from leaves of *Plumbago capensis* L.




*Gas chromatography-Mass spectrometry (GC-MS) analysis*

The main compounds of the phytosterols in *Plumbago capensis* L. were identified by using IR and GC-MS. β-sitosterol, stigmasterol, campestrol and lanosterol were reported by TLC. *Plumbago capensis* L. 26 compounds were identified in GC-MS analysis. From which one compound (Z)6,(Z)9-Pentadecadien-1-ol found in highest amount (area of % 39.63), while one compound Cyclopropane, 1-(1-methylethyl)-2-nonyl- found in lowest amount (area of % 0.09).

Phytosterols are natural product of phenolic glycosides synthesized from aromatic amino acids, occur almost naturally in angiosperms. Phytosterols have also been reported to have pathological significance in plants by providing resistance to the plants against pests and insects besides physiological importance for animals. Therefore, the difference in content between free and bound forms shows their involvement at resting and active stages, thus giving higher or lower recovery of free and bound phytosterols [15].

Table 2 GC-MS profiling of phytosterols isolated from leaves of *Plumbago capensis* L.

| R. Time | Area | Area % | Compound name |
|---|---|---|---|
| 4.21 | 34322 | 2.17 | Butyl pentyl carbonate |
| 4.26 | 25114 | 1.59 | Ethanethioic acid, S-[(methylthio)methyl] ester |
| 4.43 | 253656 | 16.04 | 4[(Bicyclo[2.2.1]hepta2,5dien7yl)oxy]benzene |
| 4.54 | 109513 | 6.93 | 2-Butanone,3-chloro-4-hydroxy-1,4-diphenyl- |
| 4.61 | 21763 | 1.38 | Propanedioic acid, (bromomethyl)methy-l,bis(1,1-dimethylethyl)ester |
| 4.86 | 183445 | 11.60 | 2-Butanone,3-chloro-4-hydroxy-1,4-diphenyl- |
| 5.28 | 16394 | 1.04 | 4,6-dimethyl-2-propyl-1,3,5-dithiazinane |
| 5.38 | 1361 | 0.09 | Cyclopropane, 1-(1-methylethyl)-2-nonyl- |
| 5.46 | 13118 | 0.83 | 4-Nonene,2-methyl-,(Z)- |
| 5.91 | 4340 | 0.27 | Sydnone, 3-(phenylmethyl)- |
| 7.57 | 14195 | 0.90 | d,l-trans-4-Methyl-5-methoxy-1-(1-methoxy-1-isopropyl)cyclohex-3-ene |
| 9.45 | 13582 | 0.86 | 4,5-Octanediol |
| 12.35 | 3228 | 0.20 | À-D-Xylofuranoside,methyl 5-Omethyl- |
| 14.88 | 15652 | 0.99 | 2,2-Dimethylpropyl2,2-dimethyl-propanesulfinyl sulfone |
| 21.35 | 6587 | 0.42 | Cyclododecanepropanenitrile |
| 21.57 | 11487 | 0.73 | Oxalic acid, cyclobutyl nonyl ester |
| 23.09 | 72709 | 4.60 | 1,15-Pentadecanediol |
| 24.82 | 8963 | 0.57 | 1,7,7-Trimethyl-2-(à-cyclohexyl-benzylimino)-bicyclo(2,2,1)heptanes |
| 26.67 | 62886 | 3.98 | Methyl 6-methylheptanoate |
| 27.05 | 7678 | 0.49 | N-Vinylpyridinium bromide |
| 27.59 | 47734 | 3.02 | 9Azabicyclo[6.1.0]nonane,9,9'azobis,[1à,8à,E(1'R*,8'S*)]]- |
| 29.51 | 10565 | 0.67 | Propane,3,3-dichloro-1,1,1,2,2-pentafluoro |
| 31.93 | 6872 | 0.43 | 1H-Imidazole-1-ethanol,2-methyl-à-phenyl |
| 32.15 | 6288 | 0.40 | 3-Phenyl-4-benzoyl-7-mercaptomethyl-2,6-dioxa-3-azabicyclo[3.3.0]-7-octene |
| 32.19 | 3223 | 0.20 | 2HPyran, tetrahydro2(2,2,2trifluoroethoxy) |
| 32.78 | 626672 | 39.63 | (Z)6,(Z)9-Pentadecadien-1-ol |

## CONCLUSION

The present investigation has been done to isolate and identify phytosterols in the experimental plant using IR, and GC-MS. The presence of these bioactive compounds in selected plants lends credence to its use for welfare of mankind. It also accounts for the production of novel medicines with isolation of specific compounds.

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