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Simultaneous estimation of Beta Sitosterol and Palmitic Acid from Methanolic extract of *Caralluma Adscedens Var Fimbriata* by UV Spetrophotometry.

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ABSTRACT

The Methanolic extract was obtained by Successive soxhelet extraction method.. All these constituents have been isolated by different solvents. A simple and reproducible U. V. Spectrophotometric method for the quantitative determination of Beta sitosterol and Palmitic acid in Methanolic extract of Caralluma adscedens var fimbriata was developed and validated. The samples were prepared in methanol and methods; obey Beers-Lamberts law in concentration ranges employed for evaluation. The method was validated using parameters such as linearity, precision, limit of detection, limit of quantification and recovery as per ICH guidelines. A new simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of Beta sitosterol and Palmitic acid in methanolic extract. The result of analysis has been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence, the proposed method can be used for the reliable quantification of active marker compound in crude drug and its herbal formulations.

Keywords: Beta sitosterol, Palmitic acid, Methanolic extract of Caralluma fimbriata, U.V.

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INTRODUCTION

Caralluma fimbriata, also known as *Caralluma adscendens*, belongs to the family asclepiadacea. In Western India it is also called Ranshabar, Makad shenguli, Kullimudayan, and Shindula makadi. There are other species of Caralluma that grow in India. Among these are: C. indica, C. attenuata, C. umbellata, and C. All these varieties of Caralluma are botanically and phytochemically similar to *C. fimbriata* and regularly consumed by the native population across India. *Caralluma fimbriata* is consumed daily as a vegetable in the Kolli Hills of South India; it is used in pickles and chutney in the arid regions of Andhra Pradesh; and in Western India, *Caralluma fimbriata* is accepted as a famine food - suppressing appetite and quenching thirst. Legend has it those hunting tribes' chewed chunks of the Caralluma cactus to suppress hunger and thirst when on a long hunt. There are no adverse events reported in the Indian subcontinent over the centuries of use of *Caralluma fimbriata.* It is listed as a vegetable in The Wealth of India and in Indian Health Ministry's comprehensive compilation on medicinal plants. Key phytochemical ingredients include sitosterol, Hexadecanoic acid, Oleic acid, pregnane glycosides, flavone glycosides, megastigman glycosides, bitter principles, alkaloids, saponins, various flavonoids etc. Caralluma has become an extremely useful type of "portable food" It is even called as "famine food", since it helps fight off hunger in times of desperate need of food for those who had to travel long distances on land [1].

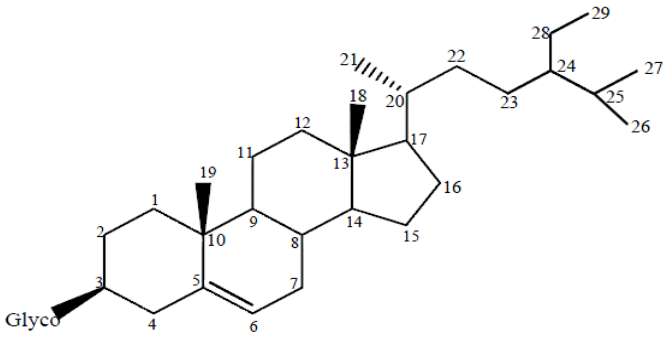

Figure 1: STRUCTURE OF β-SITOSTEROL

β-sitosterol, the principle phytosterol appears to have important immunomodulatory and anti-inflammatory activity. β-Sitosterol is one of the several phytosterols (plant sterols) with chemical structures similar to that of cholesterol. Sitosterols are white, waxy powders with a characteristic odour. They are hydrophobic and soluble in alcohol. β-sitosterol reduces cholesterol in blood and is sometimes used in treating hypercholesterolemia. β-sitosterol inhibits cholesterol absorption in the intestine. β- Sitosterol is the phytosterol with chemical structure similar to the cholesterol. It is an important nutrient in the diet meal. It is hydrophobic and soluble in organic solvents and considered as a good biomarker due to its biological activity. β- sitosterol is used as an antioxidant and as antidiabetic agent. It is also considered to be highly effective in the treatment of prostate enlargement, to boost the function of T cells and primes the immune system to function and operate more efficiently. Human liver microsome studies show that β- sitosterol inhibits the cholesterol absorption. It has shown antifertility, anti-inflammatory and antipyretic activity. β- sitosterol is proved to be chemo

preventive in the colon cancer and breast cancer cell line by inhibiting the cancer cell proliferation and along with its glucosides also plays important role in the multistage treatment of HIV, by maintaining the CD4 lymphocyte count and regulating the immune system However in the in vitro toxicological studies, chronic administration of β- sitosterol was found to be safe and non-toxic [2].

Figure 2: STRUCTURE OF PALMITIC ACID

Palmitic acid, or hexadecanoic acid in IUPAC nomenclature, is the most common fatty acid found in animals, plants and microorganisms. Its molecular formula is CH_3 $(CH_2)_{14}CO_2H$. As its name indicates, it is a major component of the oil from palm trees (palm oil, palm kernel oil, and coconut oil), but can also be found in meat, cheese, butter, and dairy products. Palmitate is a term for the salts and esters of palmitic acid. The palmitate anion is the observed form of palmitic acid at basic pH. Rats fed on a diet of 20% palmitic acid and 80% carbohydrate showed alterations in central nervous system control of insulin secretion, and suppression of the body's natural appetite-suppressing signals from leptin and insulin (the key hormones involved in weight regulation). Aluminium salts of palmitic acid and naphthenic acid were combined during World War II to produce napalm. The word "napalm" is derived from the words naphthenic acid and palmitic acid. Palmitic acid is mainly used to produce soaps, cosmetics, and release agents. These applications utilize sodium palmitate, which is commonly obtained by saponification of palm oil. Since it is inexpensive and adds texture to processed foods (convenience food), palmitic acid and its sodium salt find wide use including foodstuffs. Sodium palmitate is permitted as a natural additive in organic products.Hydrogenation of palmitic acid yields cetyl alcohol, which is used to produce detergents and cosmetics. Recently, a long-acting antipsychotic medication, paliperidone palmitate (marketed as INVEGA Sustenna), used in the treatment of schizophrenia, has been synthesized using the oily palmitate ester as a long-acting release carrier medium when injected intramuscularly [3].

MATERIALS AND METHODS

APPARATUS: Instrument used was an UV/Visible double beam spectrophotometer, SHIMADZU model 1800 (Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. An electronic analytical balance was used for weighing the sample.

REAGENTS AND MATERIALS: *Caralluma adscedens var fimbriata* plant collected from Sangli region and authentified from Krishna Mahavidyalaya ,Rethare.

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All the other chemicals and solvents were used of A.R. grade; standard β-sitosterol was procured as gift sample from Appasaheb Birnale College of Pharmacy, Sangli. Palmitic acid was procured as sample from Sigma Aldrich.

PREPARATION OF EXTRACT OF *CARALLUMA ADSCEDENS VAR FIMBRIATA:*

Soxhlet extractor was used for extraction of *Caralluma adscedens var fimbriata.* Powder of dried plant was extracted by continuous heat extraction method using solvents petroleum ether, chloroform and methanol. The extract was then concentrated and dried to obtain residue. The dried extracts weighed and the required quantity of the same was dissolved in appropriate solvents for further experiment. [4]

PREPARATION OF STANDARD STOCK SOLUTION OF β-SITOSTEROL AND PALMITIC ACID:-

The stock solution of Palmitic acid (1000µg/mL) and of β-sitosterol (1000µg/mL) was prepared by dissolving accurately 10 mg Palmitic acid in 10ml methanol and 10mg β-sitosterol in 10ml methanol and then withdrawn 1ml β-sitosterol solution and dilute to 10ml to form 100µg/ml solution of β -sitosterol. Further a series of dilutions were made with methanol.

CALIBRATION CURVE OF β –SITOSTEROL AND PALMITIC ACID:-

A series of calibrated volumetric flasks were taken and appropriate aliquots of the working standard solutions of Palmitic acid were withdrawn and diluted up to 10ml with methanol. The absorbance was measured at absorption maxima 213nm, against reagent blank prepared in similar manner without Palmitic acid. For β-sitosterol, similar procedure was applied like Palmitic acid and absorption maxima measured at 208nm, against reagent blank prepared in similar manner without β-sitosterol. Absorption maxima and Beers law limit were recorded. Data that proves linearity and obeys Beers law limit were noted. The linear correlation between these concentrations (x-axis) and absorbance (y-axis) were graphically presented. Slope (m), intercept (b) and correlation coefficient (R^2) were calculated from the linear equation (Y= mx+b) by regression [5].

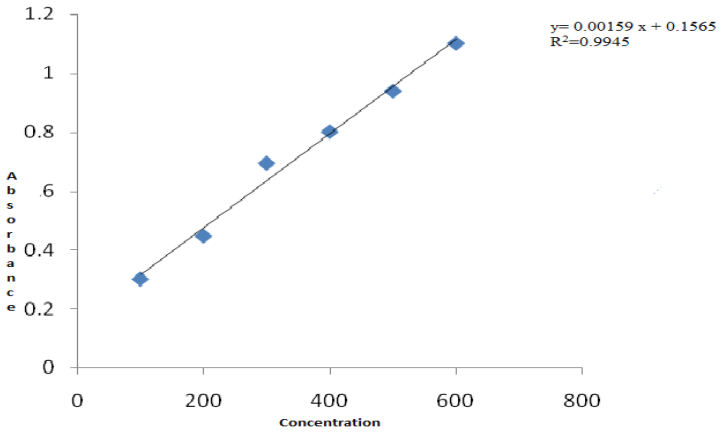

Figure 1: Calibration curve of β-sitosterol on U.V. spectrophotometer

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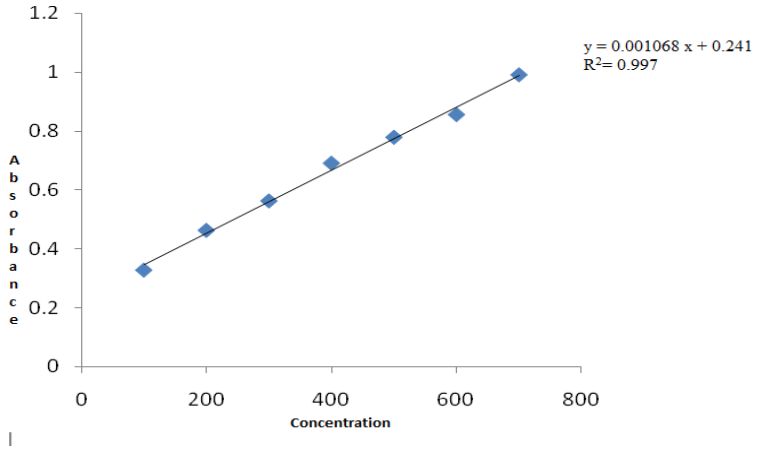

Figure 2: Calibration curve of Palmitic acid on U.V. spectrophotometer

β-sitosterol (1000µg/ml) and Palmitic acid (1000µg/ml) solutions were prepared. λmax 208 nm for β-sitosterol and 213 nm for Palmitic acid were selected as the working wavelength, at which both the drugs showed absorbance for each other. The absorptivity of both the drugs was determined at 208 nm and 213 nm respectively. A set of two simultaneous equations were formed using absorptivity values as given below, at selected wavelength. The concentration of two drugs in methanolic extract was calculated using set of two simultaneous equations. Methanolic extract showed maximum absorbance at both wavelength i.e. 208nm as λmax of β-sitosterol and 213 nm as λmax of Palmitic acid from which concentration of two drugs was calculated. [6]

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \quad (1)$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2} \quad (2)$$

Where Cx and Cy are concentrations of standard β-sitosterol and Palmitic acid in µg/ml respectively. A1 and A2 absorbance of sample solutions at 208nm and 213nm respectively. ax1 and ax2 are absorptivity of β-sitosterol at 208nm and 213nm , ay1 and ay2 absorptivity of Palmitic acid at 208nm and 213nm respectively.

The concentration of Cx and Cy in methanolic extract can be obtained by solving equation (1) and (2). The validity of above equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at two wavelengths and calculating concentration of two components.

VALIDATION OF DEVELOPED METHOD [7]

LINEARITY AND RANGE

The standard stock solution containing 1000µg/ml each of β-sitosterol and 1000µg/ml each of Palmitic acid was further diluted to get linearity concentration 100-700µg/ml for β-sitosterol and 100-600µg/ml for Palmitic acid. Each concentration was analysed in triplicates. Calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis.the relation between drug and its absorbance is expressed by equation y= mx + c, where m= slope, b= intercept.

LIMIT OF DETECTION AND LIMIT OF QUANTIZATION

LOD and LOQ of the drugs were derived by calculating signal-to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ) using following equation designated by ICH guidelines. The residual standard deviation of regression line or standard deviations of Y intercept of regression line was used to calculate LOD and LOQ.

$$LOD = 3.3 \times \frac{D}{S}$$

$$LOQ = 10 \times \frac{D}{S}$$

Where D= standard deviation of Y- intercept of regression line

S = Slope of calibration curve

RECOVERY STUDY

It was carried out by standard addition method at three different levels. 10mg standard β-sitosterol was dissolved in 10ml methanol to form stock solution of 1000µg/ml and same procedure was done for Palmitic acid and made concentration of 1000µg/ml. Prepared the concentration of both drug 80%, 100% and 120% and the concentration of sample solution i.e methanolic extract 100%. Withdrawn 1ml from the stock solution of both the drugs and mixed into 2ml of sample solution and measured the absorbance on U.V. spectrophotometer and calculated recovery and % RSD.

PRECISION: Prepared the stock solution of both the drugs by adding 10mg of drug in 10ml methanol to form 1000µg/ml β-sitosterol and 1000µg/ml of Palmitic acid and the intraday precision were determined by estimating the corresponding response 3 times on same day for β-sitosterol and Palmitic acid where as interday precision were determined by estimating the corresponding response on three different days over a period of one week. The results were reported in terms of relative standard deviation (RSD).

RUGGEDNESS: It expresses the precision within laboratories variation like different analyst. Ruggedness of the method was assessed by spiking the standard 3 times by different analyst and measuring absorbance using same equipment.

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ROBUSTNESS: By introducing small changes in concentration the effects on the results were examined. Robustness of the method was done in triplicates levels of 100µg/ml and 500µg/ml and % RSD was calculated.

RESULT AND DISCUSSION

The proposed method was validated as per ICH guideline. The absorptivity values determined for β-sitosterol were 0.00266 (ax_1), 0.00244 (ax_2) and for Palmitic acid 0.00007(ay_1), 0.00039(ay_2) at 208 nm and 213nm respectively.

B-sitosterol and Palmitic acid obeys Beer's lamberts law in concentration range 100-600µg/ml at the λmax 208nm, 100-700µg/ml at the 213 λmax respectively. The correlation coefficient (R^2) was calculated where the (R^2) value 0.9945 for β-sitosterol and 0.997 for Palmitic acid indicate good linearity between concentration and absorbance. The estimation of β-sitosterol and Palmitic acid was carried out from Methanolic extract of *Caralluma adscedens var fimbriata.* The concentration of β-sitosterol and Palmitic acid in extract was found to be 0.65µg/ml and 100µg/ml**.** All the values obtained by different validation parameters are shown in table no-1.

The validity and reliability of proposed method are assessed by recovery studies. Sample recovery for both the method is good agreement, which suggest non interference of other extracted content in estimation. Precision is determined by studying the interday and intraday precision. In both intra and inter day precision study for both the methods % RSD are not more than 2.0 % indicates good interday and intraday precision [8].

VALIDATION PARAMETERS

Parameters		Methods	
Drugs		β-sitosterol	Palmitic acid
Wavelength range (nm)		208	213
Beer's law limit (µg/ml)		100-600µg/ml	100-700µg/ml
Slope (m)		0.001595	0.001068
Intercept (b)		0.156	0.241
Correlation coefficients(r^2)		0.994	0.997
Accuracy (recovery) (n=3)	I	95.00 ± 0.002	98.75 ± 0.002
	II	96.00 ± 0.006	96.00 ± 0.002
	III	99.16 ± 0.003	99.58 ± 0.001
Precision(% RSD) n=3)	Interday	83.96 ± 0.202	90.72 ± 0.281
	Intraday	84.03 ±0.476	89.97 ±0.873
OD µg/ml		134.48	111.23
LOQ µg/ml		407.52	337.07

Table 1: Validated parameters for β-sitosterol and Palmitic acid

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RECOVERY STUDY-

Drug	Amount of drug taken (µg/ml)	Amount of drug added %	%mean recovery ± S.D (n=3)
β-sitosterol	100	80	95.00 ± 0.002
		100	96.00 ± 0.006
		120	99.16 ± 0.003
Palmitic acid	200	80	98.75 ± 0.002
		100	96.00 ± 0.002
		120	99.58 ± 0.001

CONCLUSION

Development and validation of spectrophotometric method for the estimation of β-sitosterol and palmitic acid in methanolic extract of *Caralluma adscedens var fimbriata* could be used as valuable analytical tool in routine analysis. U.V. spectrophotometric estimation of active marker compound highlights assurance of batch uniformity and integrity of product manufactured. Estimation of β-sitosterol and Palmitic acid by U.V. spectrophotometric can be used as one of the appropriate analytical method for standardization of certain plants which contains the same markers. U.V. detection of such compound is primary screening for further analysis of same by chromatographical technique.

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