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Research Article

ISOLATION AND CHARACTERIZATION OF PHYTOSTEROLS FROM BUTEAMONOSPERMA (LAMARCK.) KUNTZE

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ABSTRACT

In these decades, drastic attention in the natural products especially mentioned Ayurvedas and other conventional systems of medicines has been observed. Drugs obtained from plant have been measured much safer and possess a tremendous utility in the treatment of various diseases. Medicinal plants are called molecular factory, as they have ability to produce vastrange of compounds derived from them thus termed as "*Bioactive compounds*". The plants bears different phytochemicals known to early civilizations for various curative properties. Researches in these area sare assisting in depth knowledge about the mechanisms, their role and formation of secondary metabolites and nutraceuticals of importance. *Buteamonosperma* is significant medicinal plant having several medicinal values like antimicrobial, antioxidant and anticancer agents. It is an important arid zone flora normally consumed as herbal drugs for treatment in pain and inflammation, including othermetabolic disorders such as diabetes and obesity.During present investigations,phytosterols was characterized and quantified *in vivo*from *B. monosperma*. Chromatographic and spectral studies were used to confirm Phytosterols in which ß-sitosterol, campesterol and stigmasterol were confirmedthrough TLC, IR and GC-MS. GC-MS profiling showed presence of 49 various compounds.It was observed that maxium area was occupied by Hexadenoic acid (54.4%).

INTRODUCTION

Now these days people areattracting towards natural system. The major population is using the natural products to fulfill the daily requirements. The advanced nations are standing belonging on bioactive compounds or using pharmaceuticals based on natural products (Gbile,1986).

Phytosterolsare triterpeneswhich are vital ingredient of plant membranes, and free phytosterolsdispense to alleviate phospholipid bilayers in plant cell membranes same to cholesterol role in animal cell membranes(Moreau et al., 2002).They havesimilarchemical constituentof cholesterol which are accumulated in the small intestine to decrease cholesterol level. Since they have minute capacity of accumulation and are generally present in healthy diets, enhancing the consumption ofphytosterolsmay be a rational way to decrease cardiovascularrisk (Ostlund, 2002). There are reports thatconsumption of these sterolsnearly 1.8 grams/dayreduces LDL-cholesterol, to about 8.8%which are main cause for cardiac arrest (Berger et al., 2004). Due to this marvelousadvantage many food manufacturers and supplement developers are laying emphasis onphytosterolsas form of product to benefit both consumers andto assist a healthy supplements.

Buteamonosperma (palas) is a deciduous tree placed under familyfabaceae-papilioneae. It is called 'Flame of the Forest' and Bastard Teak(Kirtikar and Basu, 1935). They are categorizedin theprime families of angiosperms, bearing 630 genera and 18000 species.It is cosmopolitan in Asian continent , mainly in Indo-gangetic plains(Chopra *et al.,* 1958).It has been reported that this plant is worshipped as ,'God of Fire'. Flowers are delivered as a substituent of blood in sacrifice ceremony to goddess Kali(Ambasta, 1994).The plant bears various medicinal uses like anticonvulsive (Kasture*et al.,*2000), anti-inflammatory (Mengi*et al.* 1995), hypoglycemic (Somani*et al.*2006),anti helmintic(Iqbal*et al*., 2006)

MATERIALS AND METHODS

Identification and Collection of plant resources

Various plant parts (Leaves, flowers, Seeds and Roots) of *Buteamonosperma*were collected from Amer, Delhi road

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Jaipur, India. The plant samples were taxonomically recognized and confirmed by Department of Botany, University of Rajasthan(RUBL 211650), Jaipur. The plantpartswere washed, shade dried and grinded to powder. The powdered samples wereretainedin air tight containersforfurther use.

Extraction

Plant material (20g) was air dried and 30% HCl (v/v) was added for 4 h. Every sample was treated with distilled water till pH reached to neutral and was dried further. Benzene was added againfor 24 hr. The filtrate received dried*in vacuo*.The isolated samples were mixed in benzene for chromatographic studies(Kaul and Staba, 1968).

Thin layer chromatography (TLC)

Silica gels G coated with glass plates were used. Each sample was co-chromatographeddifferently with standard sterols as reference. These plates were kept in capped bottles, saturated with solvent system of Hexane: Acetone: 8:2 (Fazli and Hardman, 1968).Various solventslike benzene and ethyl acetate (85:15),(Heble et al., 1968) benzene: ethyl acetate (3:1),were used but hexane: acetone (8:2) was found to be best. Further these plates were air dried and imagined in UV light and fluorescent marks resembling with standards marker. These developed plates were treated with 50% sulphuric acid (BennetandHeftmann, 1962) and warmed at 110^0 C for 10 min.

Preparative thin layer chromatography

PTLC was done using same above plates with elevated density along with the standard markers. These plates were saturated in hexane: acetone (8:2), air dried and tested under UV light. Each spot matching with that of standard marker along with isolated compound was spotted,worn out from plates, and eluted with chloroform. The reactions were before crystallization, and further their mp, mmp were noticed down. These UV and IR spectral studies were also done.

RESULT AND DISCUSSION

Three sterols were spotted which were observed in variousplant parts on thin layer chromatography. The R_f values of the spots matched with authentic standards were identified as β-sitosterol, stigmasteroland campesterol. Among the differentsolvent tested best results were obtained in the solvent systemof Hexane: Acetone (8:2) with R_f values viz., β-sitosterol, 0.89; stigmasterol, 0.83 and campesterol, 0.23. The qualitycolours were also observed when TLC plates were treated with 50% sulphuric acid (β -sitosterol-Purple brown; stigmasterol- Gray; campesterol- Blue)corresponding to their authentic samples.

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^{o}C; stigmasterol- 167-169^{o}C and campesterol-157-158^{o}C). The characteristic peaks of IR spectra of isolates (β-sitosterol, stigmasterolandcampesterol) were also found to be super imposable with the IR spectra of reference compounds(Table 1 and Fig.1-3). Quantitative analysis showed that maximum amount was present in seeds (Table 2).A number of 49 spectral peaks was detected in GC-MS analysis as shown (Fig. 4 andTable-3). Sterols are precursors for plant steroids, which are pharmaceutically significant category of compounds, as sex hormones, corticosteroids and oral contraceptives.

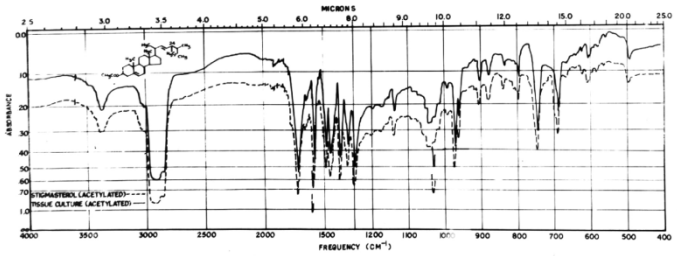

Fig 1 Infra-red Spectra of Isolated and Standard Stigmasterol

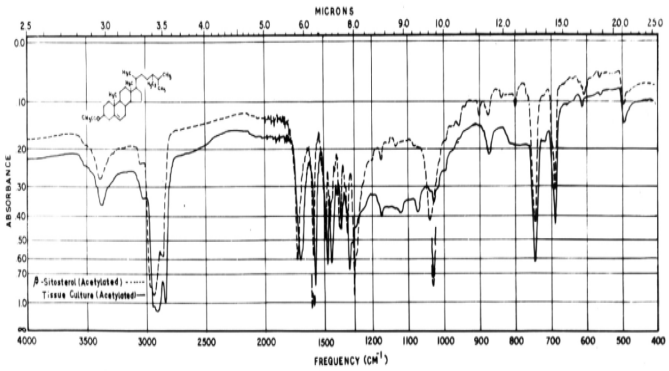

Fig 2 Infra-red Spectra of Isolated and Standard β-sitosterol

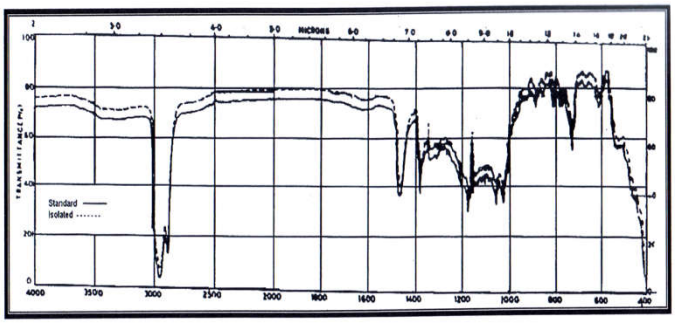

Fig 3 Infra-red Spectra of Isolated and Standard Campesterol

Table 1 Chromatographic appearance andphysio chemical nature of Isolated Phytosterols

Isolated Compounds	R_fValue			Color After Spray		M.P. (^{o}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	167-69	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

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

Table 2 Quantification of sterols isolated (mg/gdw) from various plant parts of *B. monosperma*

Plant parts	β-sitosterol	Stigmasterol	Campesterol	Total (mg/g.dw)
Flower	0.27	0.16	0.08	0.51
Seeds	0.48	0.34	0.11	0.93
Leaves	0.23	0.20	0.11	0.54
Roots	0.19	0.10	0.04	0.33

According to Stumpf and Conn (1981) the meristematic part of the plant is most active in sterol synthesis. As the tissue ages, the rate of biosynthesis of metabolites decreases, but the sterols and alkaloids on the contrary constamtlyrise until the plant starts to senescence. This increase in sterols content may be due to a loss in primary metabolites such as carbohydrates, protein etc without a corresponding loss in sterols (Grunwald, 1981).

Table 3 Profiling of phytosterols by GC-MS isolated from seeds of *Buteamonosperma*

R.Time	Area %	Compound Name	MF	M.Wt	Compound nature
4.70	0.05	2,3,4Trimethyl1pentanol, trimethylsilyl ether	$C_{11}H_{26}OSi$,	202	Ether
5.65	1.43	N(4Chlorobenzenesulfonyl) azetidin3one	$C_9H_8C_lNO_3S$	245	Keto group
19.75	0.13	Phthalic acid, hex3yl isobutyl ester	$C_{19}H_{28}O_4$	320	Ester
20.18	2.06	Pentadecanoic acid, 14methyl, methyl ester	$C_{17}H_{34}O_2$	270	Acidic
20.94	54.45	nHexadecanoic acid	$C_{16}H_{32}O_2$	256	Alkaneoic acid
22.41	0.62	LCysteine sulfinic acid	$C_3H_7NO_4S$	153	acidic
22.51	1.61	1Hexyl2nitrocyclohexane	$C_{12}H_{23}NO_2$	213	Alkane
22.79	1.06	Tetradecanoic acid, 10,13dimethyl, methyl ester	$C_{17}H_{34}O_2$,	270	ester
23.23	22.01	Oleic Acid	$C_{18}H_{34}O_2$,	282,	Fatty acid
23.46	10.55	1Decanol, 2hexyl	$C_{18}H_{34}O_2$	282	Alcohol
23.88	0.07	2Heptadecenal	$C_{17}H_{32}O$	252	Aldehyde
24.20	0.05	Galalidooctose	$C_8H_{16}O_8$,	240	Carbohydrates
24.63	0.08	4Pentadecanone	$C_{15}H_{30}O$	226	Keto
26.16	0.11	1[à(1Adamantyl) benzylidene]thiosemicarbazide	$C_{18}H_{23}N_3S$	313	carbide
27.17	0.03	4Cyclohexene1,2dicarboxylic acid, 4chloro, bis(trimethylsilyl) ester	$C_{14}H_{25}ClO_4Si_2$	348	ester
27.37	0.52	Trimethyl(3,3difluoro2propenyl) silane	$C_6H_{12}F_2Si$,	150	Alkane
28.03	0.26	1[à(1Adamantyl) benzylidene]thiosemicarbazide	$C_{18}H_{23}N_3S$,	313,	carbide
28.13	0.03	4Cyclohexene1,2dicarboxylic acid, 4chloro, bis(trimethylsilyl) ester	$C_{14}H_{25}ClO_4Si_2$	348	ester
28.79	0.03	1,1Dithiophenyl2ethylcyclobutane	$C_{18}H_{20}S_2$,	300	Alkane
29.05	0.26	Phenanthridinium, 5,6dimethyl, iodide	$C_{15}H_{14}IN$,	335	Alkylgroup
29.63	0.05	3,6Dioxa2,7disilaoctane, 2,2,7,7tetramethyl	$C_{16}H_{30}O_3Si_2$	326	Alkane
30.02	0.07	Hexestrol, diTMS	$C_{24}H_{38}O_2SI_2$,	414	sterol
30.31	0.28	3,6Dioxa2,7disilaoctane, 2,2,7,7tetramethyl3[(2methylphenoxy) methyl]	$C_{16}H_{30}O_3Si_2$,	326	Alkane
30.78	0.19	N(Methylsulfonyl)N, Obis(trimethylsilyl)hydroxylamine	$C_7H_{21}NO_3SSi_2$	255	Amine
31.58	0.25	4Methyl2,4bis(4'trimethylsilyloxyphenyl) pentene1	$C_{24}H_{36}O_2Si_2$,	412	Alkene
31.67	0.51	LProline, 4hydroxy1(trifluoroacetyl), butyl ester, trans	$C_{11}H_{16}F_3NO_4$,	283	Amino acid

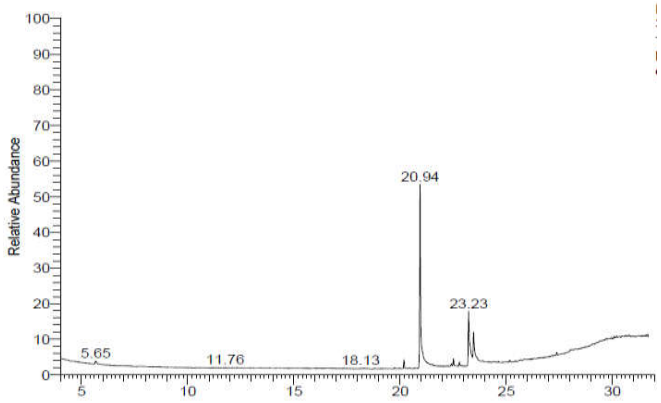

Fig 4 Gas Chromatography-Mass Spectrometry (GC-MS)

CONCLUSIONS

The present investigation has been done to isolate and identify sterols found in *Buteamonosperma*using IR and GC-MS. The occurrence of these natural product in *Buteamonosperma*lends credence to its use for welfare of mankind. It also accounts for the synthesis of innovativemedicines with isolation of specific compounds.

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