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IN THE PRESENT STUDY AN ATTEMPT WAS MADE TO ISOLATE AND CHARACTERIZE THE PHYTOCONSTITUENTS FROM AND UTILIZE THEM AS MARKER COMPOUNDS. POWDERED ROOT OF THE DRUG WAS EXTRACTED AND THE BUTANOLIC EXTRACT WAS USED FOR THE ISOLATION PROCESS. ISOLATION OF THE PHYTOCONSTITUENTS WAS DONE BY COLUMN CHROMATOGRAPHY USING GRADIENT ELUTION WITH DIFFERENT STATIONARY PHASES AND MOBILE PHASES OF VARYING POLARITY. TWO COMPOUNDS WERE ISOLATED AND CHARACTERIZED BY 438 SPECTROSCOPY. 62) METHOD WAS DEVELOPED TO ESTIMATE THE ISOLATED TWO COMPOUNDS. THE SIMPLICITY OF ISOLATION AND 62) ANALYSIS OF THE COMPOUNDS MAY BE UTILIZED AS MARKERS IN STANDARDIZATION OF PREPARATIONS CONTAINING 1
 (3 MARKER) COMPOUNDS) COLUMN) CHROMATOGRAPHY, 2) 9HATAVARIS 3 62) . 62) .fl. 438, (fl.) 438

INDIA HAS A RICH MEDICINAL PLANT FLORA OF SOME 438 SPECIES OF WHICH AT LEAST 438 SPECIES ARE USED COMMERCIALY ON A FAIRLY LARGE SCALE. SPARAGUS RACEMOSUS IS ALSO ONE OF THE COMMONLY USED MEDICINALLY IMPORTANT HERBS. SPARAGUS RACEMOSUS "ZILLACEAE" COMMONLY CALLED AS 9HATAVAR IS AN HERB GROWING WIDELY THROUGHOUT INDIA. SPARAGUS RACEMOSUS IS A PERENAL CLIMBER WITH FASCICLED FINGER LIKE CLUSTERED TUBEROUS ROOTS PRODUCING CUPICIOUS AMOUNT OF

L

' NUMBER OF PHYTOCONSTITUENTS BELONGING TO DIFFERENT CLASSES HAVE BEEN ISOLATED AND CHARACTERIZED FROM ' SPARAGUS RACEMOSUS USING AQUEOUS ' HYDROALCOHOLIC SOLVENTS : THE ROOTS CONTAIN FOUR STEROIDAL SAPONINS LIKE 9-HATAVARIN / ~ / < , ISOFLAVONIDS , ASPARAGAMINE ' ' A POLYCYCLIC ALKALOID' , RACEMOFURAN , \$, fifi'DIHYDROPHENANITHENE DERIVATIVE RACEMOSOL , GLYCOSACCHARIDE AND MUGIAGEI * IOSGENIN AND QUERCETIN '4' GLUCURONIDE ARE PRESENT IN THE LEAVES 9-TOSTEROL , STIGMATEROL , SARASAPOGENIN , STOSTEROL BETA ~* ~ GLUCOSIDE , TWO SPIROSTANOLIC AND FUROSTANOLIC SAPONINS ARE PRESENT IN THE FRUITS 9-HATAVARIN / AND / < ARE THE MAJOR STEROIDAL SAPONIN GLYCOSIDES¹² "1

3 MARKERS CONSTITUTE CHEMICALLY DEFINED CONSTITUENTS WHICH CAN SERVE AS A POWERFUL TOOL FOR STANDARDIZATION OF FINISHED FORM OF HERBAL PREPARATIONS . 6.2) ANALYSIS OF SELECTED SAPOGENINS IN ' SPARAGUS RACEMOSUS HAS BEEN REPORTED¹¹ . HOWEVER NO OTHER COMPOUNDS OF 9-HATAVAR HAVE BEEN REPORTED AS MARKERS . HENCE THERE IS A NEED TO ISOLATE , PURIFY AND QUANTIFY THE PHYTOCONSTITUENTS AS MARKER COMPOUNDS FROM ' SPARAGUS RACEMOSUS IN ORDER TO STANDARDIZE ITS EXTRACTS AND FORMULATIONS

IN THE PRESENT STUDY AN ATTEMPT WAS MADE TO ISOLATE , CHARACTERIZE AND QUANTIFY THE CHEMICAL CONSTITUENTS FROM THE EXTRACTS OF ROOTS OF ' SPARAGUS RACEMOSUS USING SIMPLE CHROMATOGRAPHIC TECHNIQUES . 6.2) PROFILING WAS ALSO DONE IN ORDER TO CHARACTERIZE THEM AS MARKERS

: THE DRIED PLANT ROOTS OF ' SPARAGUS RACEMOSUS WERE OBTAINED FROM 4 NATURAL REMEDIES 6<: 2 * , (ANGALORE AND AUTHENTICATED BY 4.9)' / 8 , * ELHI ' < OJHER NO 4.9)' / 8. 3 / ē f i f i f i v , † / ē † " & THE ROOTS WERE THEN SUN DRIED , POWDERED AND STORED IN AIR TIGHT CONTAINERS FOR FURTHER USE

: THE ROOTS OF ' SPARAGUS RACEMOSUS WERE SUBJECTED FOR EXTRACTION USING STATIC EXTRACTOR : THE POWDERED ROOT WAS REFLUXED WITH FRESH METHANOL "1% DRUG TO SOLVENT RATIO" FOR ONE AND HALF HOUR AT TEMPERATURE NOT EXCEEDING ! ž fi) AND THE EXTRACT WAS STRAINED THROUGH MUSLIN CLOTH TO OBTAIN THE FIRST EXTRACT : THE MARC WAS AGAIN SUBJECTED FOR SECOND TIME EXTRACTION AND THE EXTRACT OBTAINED WAS STRAINED THROUGH MUSLIN CLOTH TO YIELD 2ND EXTRACT : THE SAME PROCEDURE WAS

FOLLOWED FOR THIRD WASH TO OBTAIN THE 3RD EXTRACT : THE FIRST , 2ND , 3RD EXTRACTS WERE COMBINED TOGETHER AND CONCENTRATED TO OBTAIN A THICK PASTE

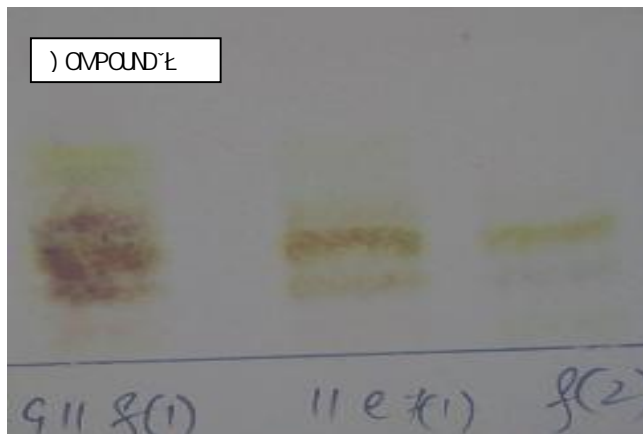
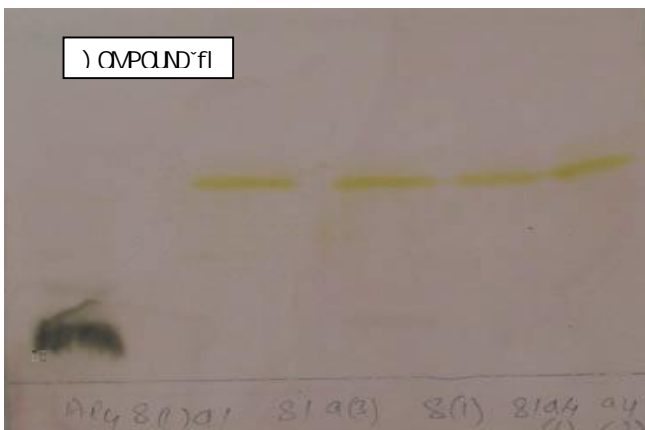
: THE METHANOLIC EXTRACT WAS FRACTIONATED WITH BUTANOL AND WATER TO GET SAPONIN RICH FRACTION , FURTHER THE BUTANOLIC EXTRACT WAS USED FOR ISOLATION BY COLUMN CHROMATOGRAPHY USING GRADIENT ELUTION METHOD

* DIFFERENT SOLVENTS COMBINATIONS WERE TRIED FOR DEVELOPING A : 2) SYSTEM IN ORDER TO IDENTIFY THE CONSTITUENTS IN THE EXTRACT AND THOSE SHOWING MAXIMUM SEPARATION WERE SELECTED AS THE MOBILE PHASE : THE FOLLOWING SOLVENTS EXHIBITED MAXIMUM SEPARATION , HENCE WERE USED FOR THE DEVELOPMENT OF THE : 2) SYSTEM

- f1i) CHLOROFORM% METHANOL% WATER " ! ž % fi % ž "
- ē1) CHLOROFORM% 2 ACETIC ACID % METHANOL% WATER " ! ž % ē % fi % ž "
- †1) CHLOROFORM% METHANOL% WATER " " % ē ! % fi ž "
- ž1) CHLOROFORM% METHANOL% WATER " " % % fi ž "

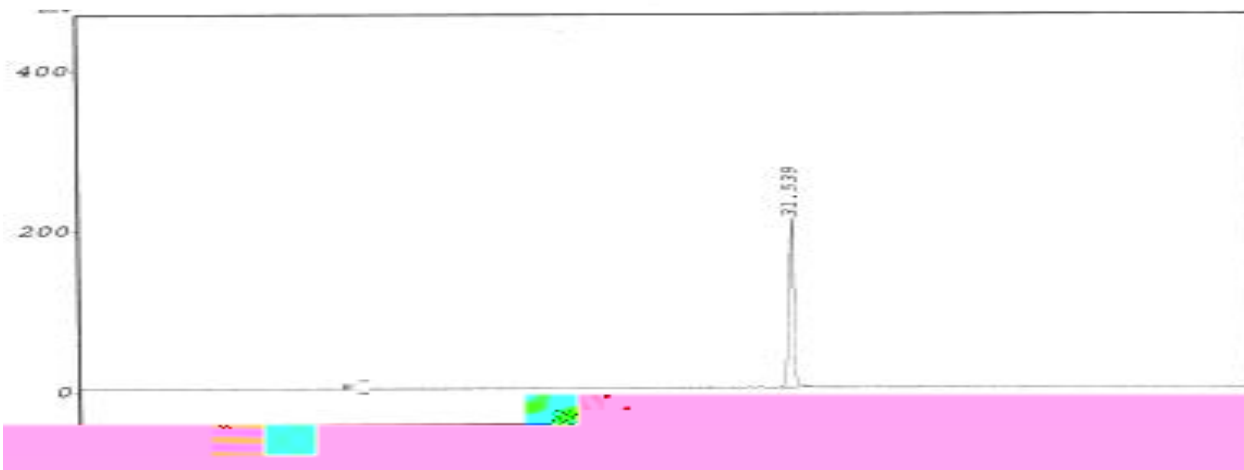
#&3+&%)4&-;!5/) ~!5-7'/'i+~fi"//Ei"/i fi

' i ~ " " "z% ~' Z' i fiL \$# ~ f#f'&fV

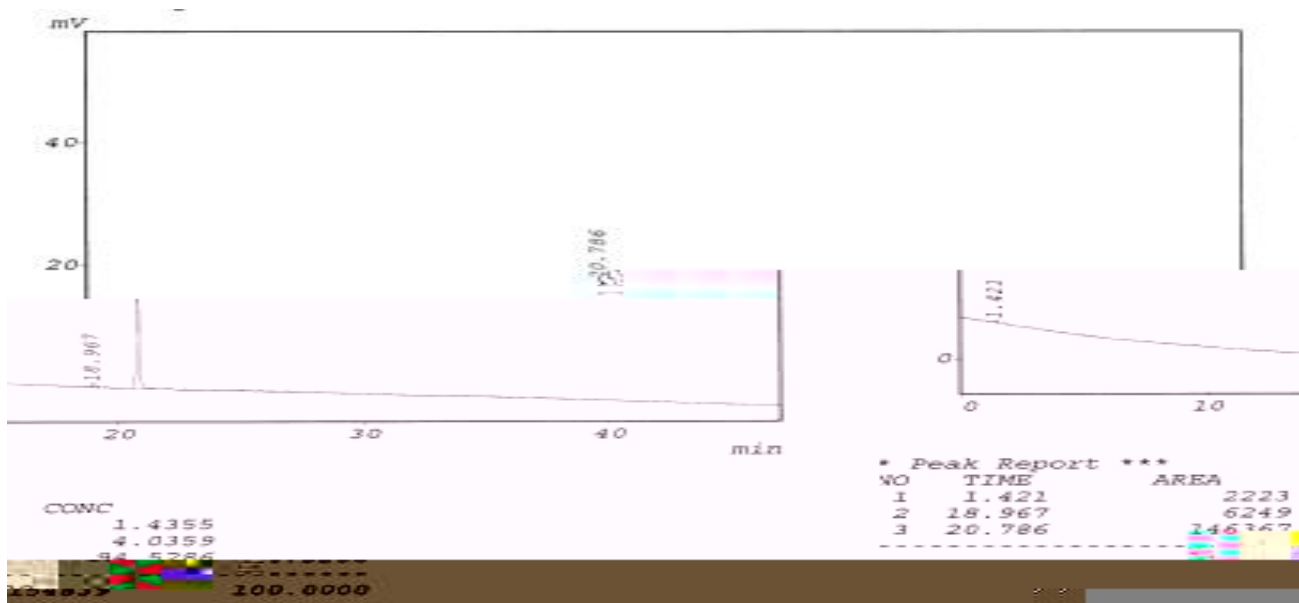


SHOWING DISTINCT SPOTS OF COMPOUND 'fi'

SHOWING DISTINCT SPOT OF COMPOUND 'E' 'R'

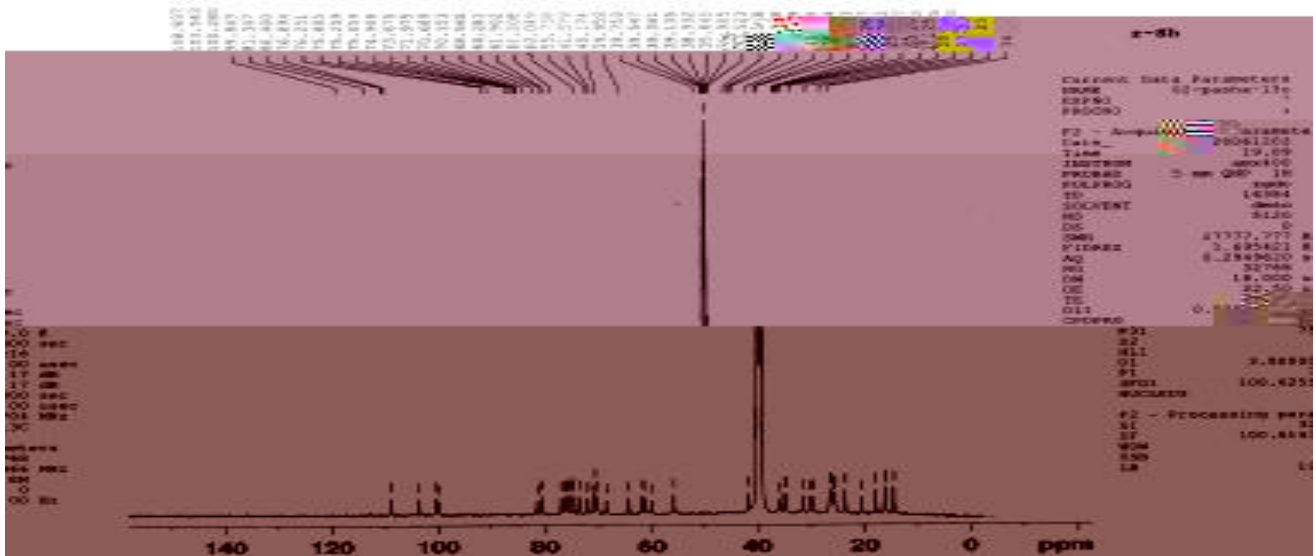


. 62 CHROMATOGRAM OF COMPOUND 'fi'



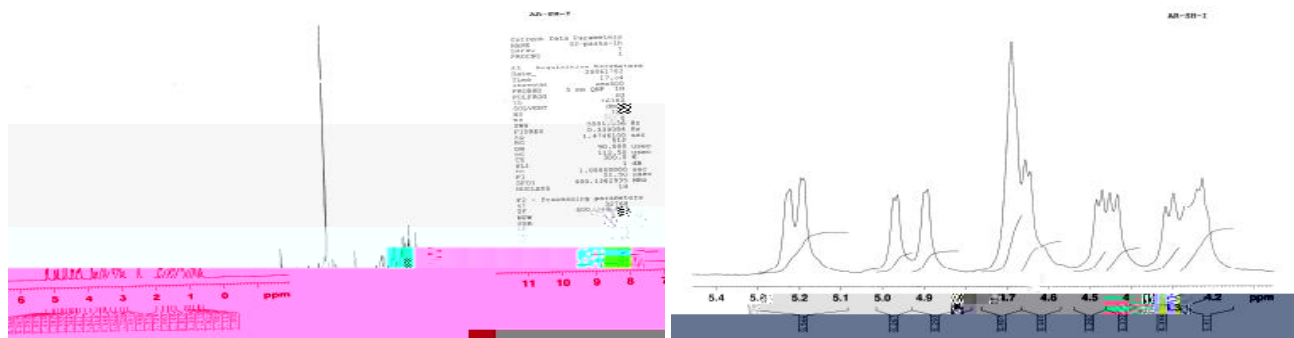
. 62 CHROMATOGRAM OF COMPOUND 'E'

#&3+&%)4&-'15/) '15-7/'it'É'fi'//É'//fi fi

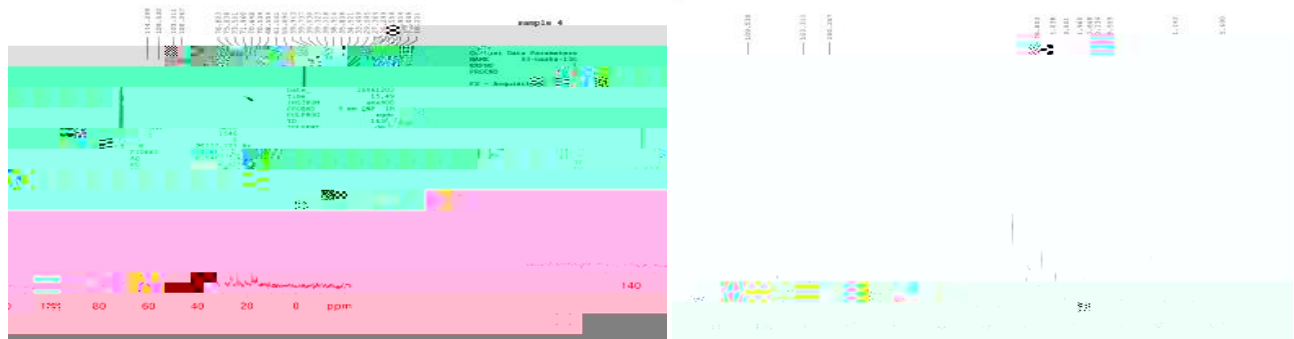


ii) 438 OF COMPOUND fi

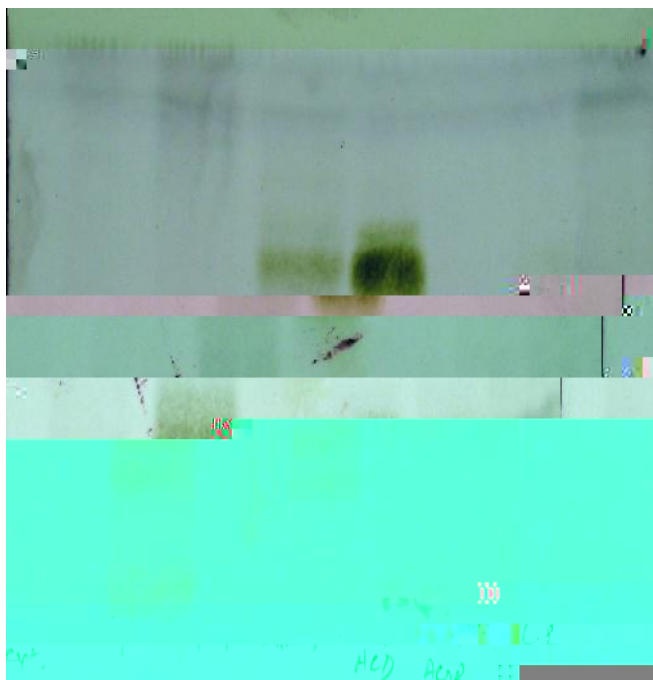
#&3+&%)4&.!5/) ~!5-7'/'if"t~f"i"///t~f"/f fi



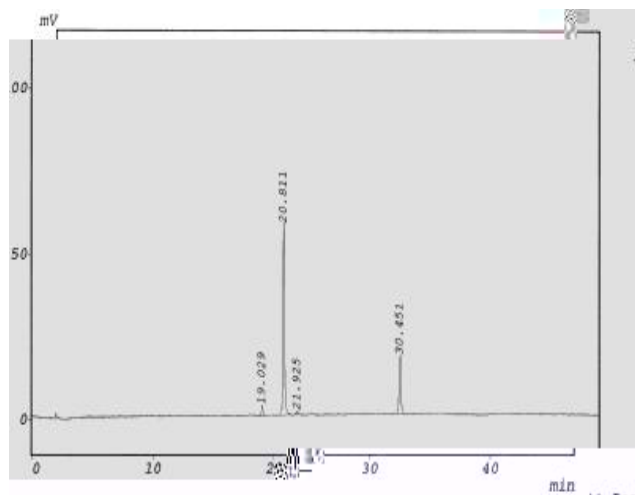
fil_438CF) COMPOUND~fil



fil_438CF) COMPOUND~fil

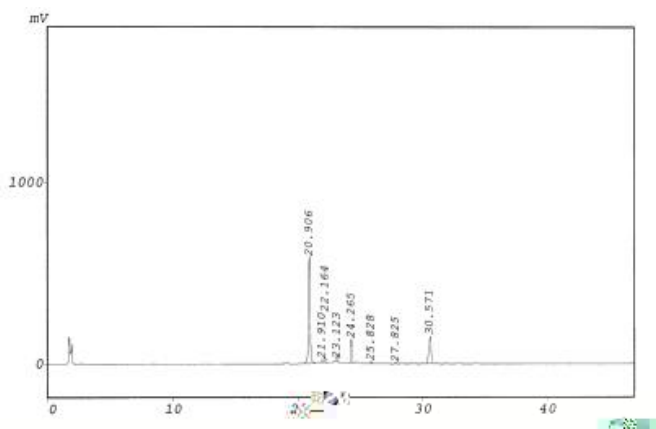


2) CHROMATOGRAM OF DIFFERENT EXTRACTS ALONG WITH ISOLATED COMPOUNDS
 SOLVENT SYSTEM CHLOROFORM:METHANOL:WATER = 8:1:1
 DETECTION WITH NSALDEHYDE REAGENT



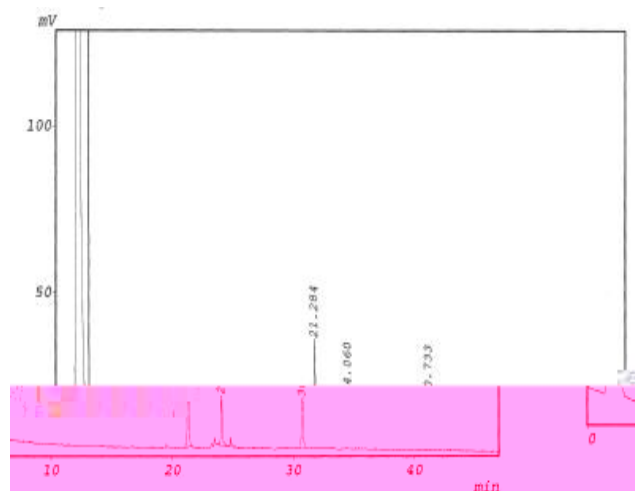
TIME	AREA	CONC
19.029	21150	2.9959
21.925	7739	1.0811
30.811	507459	70.8928
30.451	179169	25.0302
715812		100.0000

62) CHROMATOGRAM OF THE MIXTURE OF COMPOUND fi AND COMPOUND t



TIME	AREA	CONC
20.906	532008	60.7371
21.910	119191	1.3608
22.164	196299	2.2411
23.123	289326	3.3032
24.265	798629	9.1177
25.828	81390	0.9292
27.825	32497	0.3710
30.571	1921742	21.9400
8759081		

62) CHROMATOGRAM OF THE BUTANOLIC EXTRACT SHOWING THE PRESENCE OF MARKERS COMPOUNDS fi t



TIME	AREA	CONC
21.284	260924	49.7605
24.060	116665	22.2492
30.733	146770	27.9903
324359		100.0000

62) CHROMATOGRAM COMMERCIAL ROOT EXTRACT SHOWING THE PRESENCE OF MARKERS COMPOUNDS fi t

SAPOGENINS AS WELL AS TWO TERTIARY METHYL GROUPS? flififi "O
!i# . Z" AND flififi fi "O!i# . Z"@:HE PRESENCE OF ANOTHER
TERTIARY METHYL GROUP AT fi! " PPM "D" O!i# . Z" AND FOUR
ANOMERIC PROTONS SIGNALS AT ži#ž "D, O"i# . Z". žiž! "D, O
"i#" ži\$Ł "BRS" AND ži#fi "D, O"i! . Z" SUGGESTED THE
PRESENCE OF FOUR MONOSACCHARIDES INCLUDING ONE
DEOXYHEXOSE (BASED ON THIS THE STRUCTURE WAS FOUND TO HAVE
MOLECULAR FORMULA) žfi. #! 5 Ł! I

AUTHENTICATED DRIED ROOTS, STEM AND LEAVES OF SPARGANUS
RACEMOSUS WERE SUBJECTED TO EXTRACTION AND THE EXTRACTS
WERE SUBJECTED TO GC: THE ROOT EXTRACT AND BULBILIC
EXTRACT WERE FOUND TO CONTAIN COMPOUND fi AND COMPOUND Ł
AS SHOWN IN FIGURE NO\$! :HE . 62) OF THE MIXTURE OF
COMPOUND fi AND COMPOUND Ł WAS CARRIED OUT AND THE
CHROMATOGRAM IS SHOWN IN, FIGURE NO\$ifi! ' ILTHE EXTRACTS WERE
SUBJECTED TO . 62) :HE CHROMATOGRAMS ARE SHOWN IN, FIGURE
fifi"fiŁ. ENCE RESULTS SUGGESTED THAT THE . 62) ANALYSIS OF THE
EXTRACTS ALSO INDICATED THE PRESENCE OF THESE TWO MARKER
COMPOUNDS

GHATAVAR IS USED IN TRADITIONAL SYSTEM OF MEDICINE FOR
TREATMENT OF MANY DISEASES. NUMBER OF STUDIES ON ISOLATION
OF CHEMICAL CONSTITUENTS^{ŁŁŁ} AND PHARMACOLOGICAL ACTIVITIES^{žž}
Ž! ~~GHATAVAR IS USED IN TRADITIONAL~~ GHATAVARNS HAVE BEEN ISOLATED AS BIOLOGICALLY ACTIVE
COMPOUNDS (UTTI DS TI

%%%%

COMPOUNDS (UTTI DS TI

#&3+&%)4&-;!5/) ~!5-7'/'i†~Ł~fi"//ŁŁ"/†fi