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EPICUTICULAR n-ALKANES FROM DIFFERENT PARTS OF *KLEINHOVIA HOSPITA* LINN. AND THEIR MICRO-MORPHOLOGICAL STUDIES

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ABSTRACT

n-Alkanes are widely utilized for terrestrial plant biomarkers and for the variation of chain-length abundances in modern plants around the world. The distribution of n-alkanes composition of the plant parts (leaves, flowers and seeds) of *Kleinhovia hospita* showed wide variation among themselves within the same geographical region. The n-hexane extract separately obtained from fresh mature leaves, flowers and seeds of *K. hospita* containing a thin layer of epicuticular waxes, has been analyzed for the first time by thin-layer chromatography (TLC), gas chromatography (GC) and scanning electron microscopy (SEM). The surface wax of leaves, flowers and seeds contain eighteen, nineteen and sixteen long chain hydrocarbons accounting of 94.07%, 95.92% and 93.85% respectively. The predominant n-alkanes are Hentriacontane (C_{31}), Tritriacontane (C_{33}) and Heptatriacontane (C_{37}) respectively. SEM studies of surface features of leaves, flowers and seeds of *K. hospita* as well as their respective extracted hydrocarbons showed characteristic micro-morphological architecture and chemotaxonomic significance.

KEYWORDS: *Kleinhovia hospita*, n-alkanes, epicuticular wax, GC, SEM, chemotaxonomy.

INTRODUCTION

Kleinhovia hospita Linn. (Family: Malvaceae; earlier Sterculiaceae) is an evergreen, bushy tree growing up to 20 m high. The genus is monotypic, distributed in tropical areas of Asia, Africa and Australia.[1] Many chemical constituents along with their pharmacological

activities of the plant, *K. hospita* have been detected.[2-5] The outermost layer of the plant cuticle is covered with epicuticular waxes which consist of complex mixtures of long chain aliphatic and cyclic components such as hydrocarbons, primary and secondary alcohols, aldehydes, ketones, esters, fatty acids and triterpenoids.[6, 7] Epicuticular waxes provide many functions to protect plants against UV light and uncontrolled loss of water.[8, 9] The carbon chain lengthwise distribution pattern of n-alkanes is considered as a good taxonomic marker.[10] In this communication, the hydrocarbon composition of the epicuticular wax of leaves, flowers and seeds in terms of n-alkanes along with their surface micro-morphological studies by SEM has been explored to find the utility in chemotaxonomic characterization.

MATERIALS AND METHODS

Plant materials and Chemicals

Matured and fresh leaves, flowers and seeds were collected in the morning during the month of August, September and January respectively from the Research Farm (23°53′N latitude and 83°25′E longitude), Department of Botany, The University of Burdwan, West Bengal, India. The plant was authenticated by Prof. Ambarish Mukherjee, Department of Botany, The University of Burdwan. Voucher specimens MCD1 for leaves, MCD2 for flowers and MCD3 for fruits of this plant have been deposited at the herbarium (BURD) of the Department of Botany, The University of Burdwan. All solvents used in this experiment were of analytical grades purchased from E. Merck (Mumbai, India), TLC plates (thickness 0.5 mm) were prepared with silica gel G (Merck, India) using Unoplan Coating apparatus (Shandon, London, UK) and standard hydrocarbon samples were purchased from Sigma (USA).

Wax extraction

Plant material (100g) each of leaves, flowers and seeds were initially rinsed with distilled water and dried on paper towelling. Samples were then dipped in 2L n-hexane for 45 min at room temperature. The hexane extract was passed through Whatman 41 (Maidstone, UK) filter paper, and the solvent was removed under reduced pressure. The crude extracts thus obtained were then fractionated by preparative TLC on silica gel G thin-layer chromatography (TLC) with carbon tetrachloride as the mobile phase. The single hydrocarbon band (Rf = 0.9) was identified through co-TLC studies with standard hydrocarbon samples. The band was then eluted from the layer with chloroform. The eluted band samples showed no absorption for any detectable functional group in the infrared region

and the absence of alkenes was further confirmed by argentometric TLC clearly indicating the presence of only alkanes.[11]

GC analysis of hydrocarbon samples

Purified hydrocarbon samples obtained from leaves, flowers and seeds were analysed by capillary GC on a Shimadzu Gas Chromatograph (Model: GC-2010, Shimadzu, Japan) with flame ionization detector (FID) on a split injector. For alkane analysis a Rtx 5 Ms column (Serial No. 1009701; 25 m long x 0.25 mm i.d) was used. The temperatures set for injector and detector ports were 260 ^{0}C and 270^{0}C respectively. The oven temperature program was initially at 80^{0}C for 2 minutes, and raised at 10^{0}C/min to 250^{0}C (set for 5 minutes) and then again raised at 15^{0}C/min upto 280^{0}C (set for 34 minutes). The carrier gas was nitrogen at flow rate of 30 ml/min. Components were identified and characterized by co-elution with the standard n-alkanes.

Scanning Electron Microscopic (SEM) studies

SEM of surface waxes of mature leaves, flowers and seeds as well as their respective extracted hydrocarbons were done. Each of the samples was mounted on circular aluminium stubs with double sticky tape, and coated with 20 nm of gold using an IB2 ion coater. The samples were examined and photographed using a scanning electron microscope (model S-530; Hitachi Ltd., Tokyo, Japan) at accelerating potential of 15 kV.

RESULTS AND DISCUSSION

Different plant parts (leaves, flowers and seeds) showed a wide distribution of n-alkanes ranging from C_{15} to C_{37}. Table 1 showed the isolation of pure surface wax (hydrocarbons) from 100g of each leaves, flowers and seeds that resulted into 11.60 mg, 21.80 mg and 8.56 mg respectively. The relative percentage of hydrocarbons of leaves, flowers and seeds were placed in Table 2 which revealed that the surface waxes of leaves, flowers and seeds contained eighteen, nineteen and sixteen long chain hydrocarbons respectively. The GC analyses showed a wide range of n-alkane predominance in leaf of C_{17} to C_{37} (except C_{19}, C_{21} and C_{34}) (Fig. 1) where as flower contained C_{16} to C_{37} (except C_{19}, C_{34} and C_{36}) (Fig. 2) and C_{15} to C_{37} (except C_{16}, C_{19}, C_{23}, C_{25}, C_{30}, C_{34} and C_{36}) (Fig. 3) were obtained from seed. The predominant n-alkanes Tritriacontane (C_{33}) (32.22%) and Heptatriacontane (C_{37}) (31.19%) from leaves; Hentriacontane (C_{31}) (45.57%), Tritriacontane (C_{33}) (14.90%) and Nonacosane (C_{29}) (10.16%) from flowers; Hentriacontane (C_{31}) (27.83%) and Tritriacontane (C_{33}) (24.11%) from seeds were found. The ratio of odd to that of even numbered hydrocarbons

was 5.77:1 for leaf, 4.45:1 for flower and 2.44:1 for seed (Table 3). Total n-alkane content was in the order: flower (95.92%) > leaf (94.07%) > seed (93.85%) respectively. SEM images of epicuticular wax on the upper surface (Fig. 4) and lower surface (Fig. 5) of *K. hospita* leaves show deposition of wax accumulating around and within stomata on the leaf surface. Both the upper and lower surfaces have elliptical stomata. Some dust particles along with the trichomes are also seen in both the figures. Fig. 6 and Fig. 7 show the deposition of epicuticular wax of the outer and inner surface of the flower petals. The inner portion of the flower has some regular rope like deposition of surface hydrocarbon whereas the SEM image of outer face of flower petals appears as wavy fabric. SEM study of mature seed reveals that the outer (Fig. 8) surface is closely spaced prop roots of banyan tree and inner surface (Fig. 9) is indefinite shapes of clouds like structure. The SEM images of pure hydrocarbon isolated from different parts (Figs. 10-12) reflect more or less scattered with various shapes and sizes and distinguishable from each other.

The determination of n-alkanes has been characterised for the first time from leaves, flowers and seeds of *K. hospita*. Hydrocarbon from the epicuticular waxes in higher plants comprising mainly a wide range of n-alkane homologues (C_{15}–C_{38}) with the predominance of odd number carbons atoms.[12] The higher abundances odd-numbered n-alkanes (C_{29}, C_{31}, C_{33}, C_{35} and C_{37}) indicate the chemical taxonomic evaluation of higher plants.[13, 14]

Table 1: The amount variation of extractable surface wax and surface n-alkanes obtained from different parts of *K. hospita*.

Parts of the plant	Leaves (mg/100g fresh wt.)[a]	Flowers (mg/100g fresh wt.)[a]	Seeds (mg/100g fresh wt.)[a]
Total surface wax	120.51 ± 0.41	141.12 ± 0.59	40.22 ± 0.22
Pure hydrocarbon	11.60 ± 0.17	21.80 ± 0.47	8.56 ± 0.14
Other constituents of surface wax	108.91 ± 0.14	119.32 ± 0.18	31.66 ± 0.11

[a]Values are means ± S.E., n=3

Table 2: n-Alkane constituents in the surface wax of leaves, flowers and seeds of *K. hospita.*

n-Alkanes	Leaves		Flowers		Seeds	
	Retention time (min)	Relative percentage[b]	Retention time (min)	Relative percentage[b]	Retention time (min)	Relative percentage[b]
Pentadecane (C_{15})	-	-	-	-	8.921	1.16 ± 0.04
Hexadecane (C_{16})	-	-	9.967	0.37 ± 0.02	-	-
Heptadecane (C_{17})	11.663	0.52 ± 0.04	11.662	1.31 ± 0.04	11.669	2.34 ± 0.07
Octadecane (C_{18})	12.472	1.84 ± 0.03	12.474	1.86 ± 0.04	12.477	1.95 ± 0.07
Nonadecane (C_{19})	-	-	-	-	-	-
Eicosane (C_{20})	14.716	2.43 ± 0.12	14.721	3.04 ± 0.07	14.722	4.02 ± 0.04
Heneicosane (C_{21})	-	-	15.677	0.12 ± 0.00	15.622	1.29 ± 0.06
Docosane (C_{22})	16.749	1.96 ± 0.06	16.755	3.07 ± 0.11	16.753	4.14 ± 0.11
Tricosane (C_{23})	17.796	3.85 ± 0.03	17.745	0.17 ± 0.02	-	-
Tetracosane (C_{24})	18.605	1.92 ± 0.09	18.610	2.74 ± 0.03	18.609	3.87 ± 0.04
Pentacosane (C_{25})	19.536	0.71 ± 0.04	19.543	1.50 ± 0.07	-	-
Hexacosane (C_{26})	20.496	1.66 ± 0.23	20.505	2.23 ± 0.12	20.503	3.51 ± 0.06
Heptacosane (C_{27})	21.714	0.52 ± 0.06	21.729	1.97 ± 0.09	21.726	1.42 ± 0.05
Octacosane (C_{28})	23.099	1.10 ± 0.04	23.112	1.89 ± 0.05	23.117	3.48 ± 0.05
Nonacosane (C_{29})	24.790	0.95 ± 0.12	24.834	10.16 ± 0.27	24.801	3.11 ± 0.02
Triacontane (C_{30})	26.491	1.19 ± 0.07	26.541	0.39 ± 0.05	-	-
Hentriacontane (C_{31})	27.175	7.63 ± 0.13	27.288	45.57 ± 0.14	27.190	27.83 ± 0.17
Dotriacontane (C_{32})	28.465	1.46 ± 0.07	28.490	2.00 ± 0.06	28.486	6.35 ± 0.10
Tritriacontane (C_{33})	30.028	32.22 ± 0.12	30.078	14.90 ± 0.11	30.014	24.11 ± 0.12
Tetratiacontane (C_{34})	-	-	-	-	-	-
Pentatriacontane (C_{35})	31.799	2.58 ± 0.04	31.796	0.79 ± 0.05	31.799	2.82 ± 0.02
Hexatriacontane (C_{36})	32.897	0.34 ± 0.02	-	-	-	-
Heptatriacontane (C_{37})	34.023	31.19 ± 0.15	33.990	1.84 ± 0.08	33.974	2.45 ± 0.09

[b]Values are means ± S.E., n=3

Table 3: Relative percentage of hydrocarbon and their odd-even ratio of leaves, flowers and seeds of *K. hospita.*

Parts of the plant	Leaves	Flowers	Seeds
Total n-alkanes (relative percentage)	94.07	95.92	93.85
Odd numbered hydrocarbons (relative percentage)	80.17	78.33	66.53
Even numbered hydrocarbons (relative percentage)	13.90	17.59	27.32
Ratio of Odd and Even numbered hydrocarbons	5.77:1	4.45:1	2.44:1

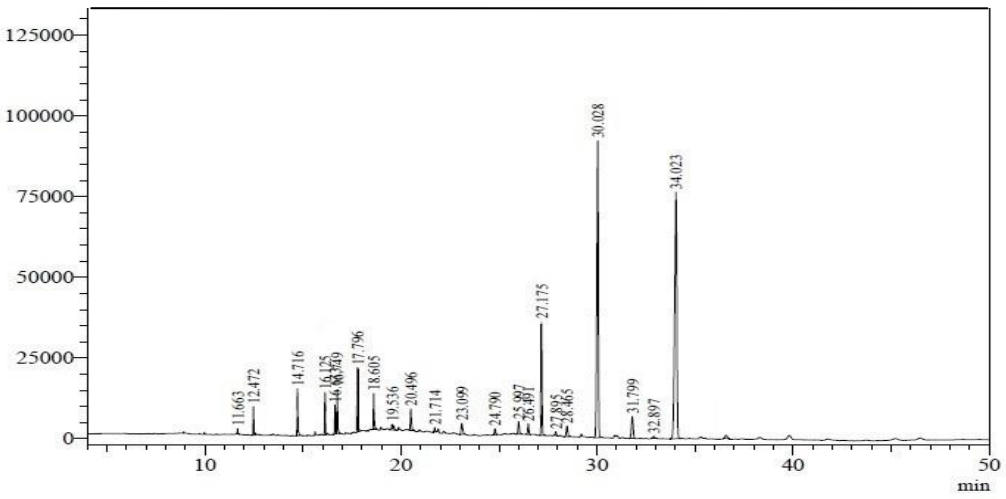

Fig. 1: GC chromatogram of purified leaf hydrocarbon of *K. hospita.*

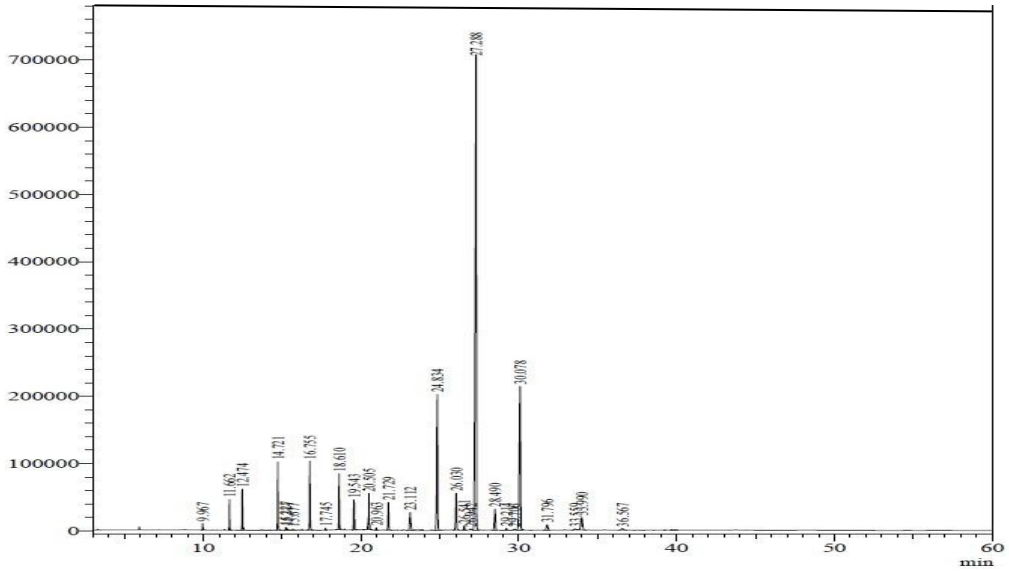

Fig. 2: GC chromatogram of purified flower hydrocarbon of *K. hospita.*

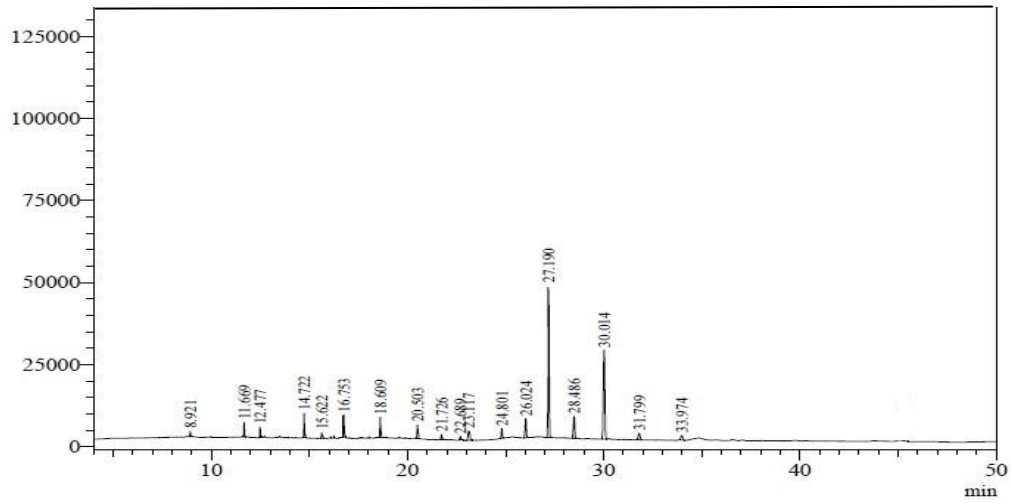

Fig. 3: GC chromatogram of purified seed hydrocarbon of *K. hospita.*

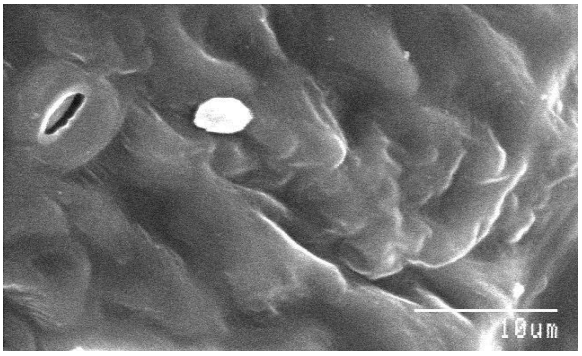

Fig. 4: SEM image of epicuticular wax pattern of upper surface of *K. hospita* leaf.

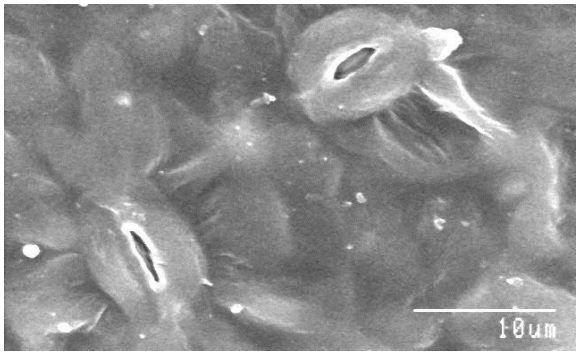

Fig. 5: SEM image of epicuticular wax pattern of lower surface of *K. hospita* leaf.

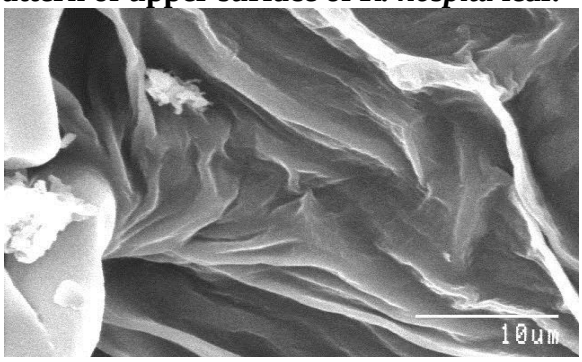

Fig. 6: SEM image of epicuticular wax pattern of outer surface of *K. hospita* flower.

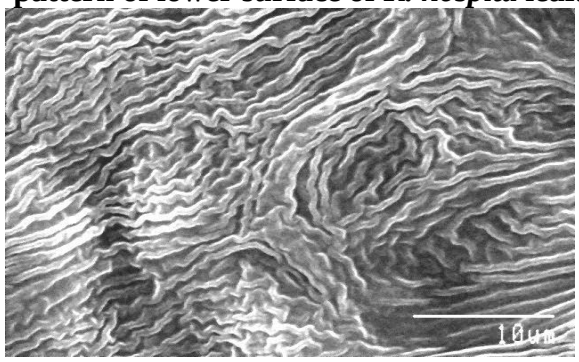

Fig. 7: SEM image of epicuticular wax pattern of inner surface of *K. hospita* flower.

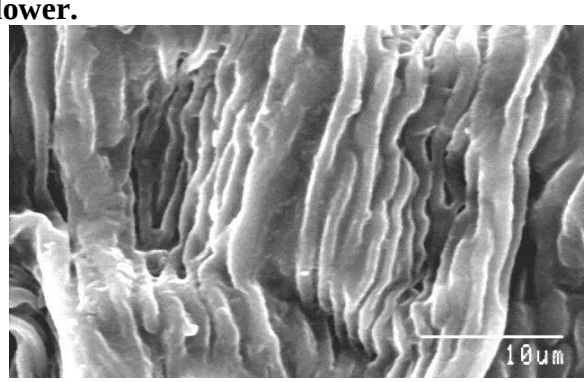

Fig. 8: SEM image of epicuticular wax pattern of outer surface of *K. hospita* seed.

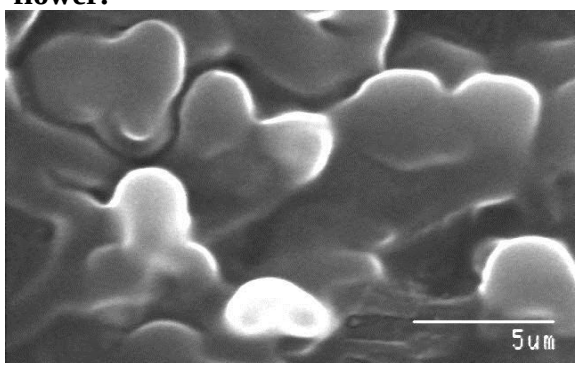

Fig. 9: SEM image of epicuticular wax pattern of inner surface of *K. hospita* seed.

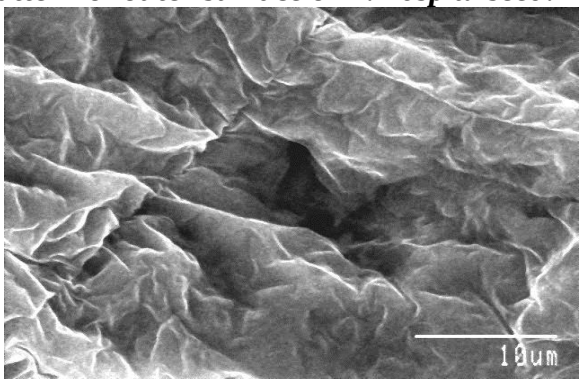

Fig. 10: SEM image of hydrocarbon isolated from crude extract of *K. hospita* leaf.

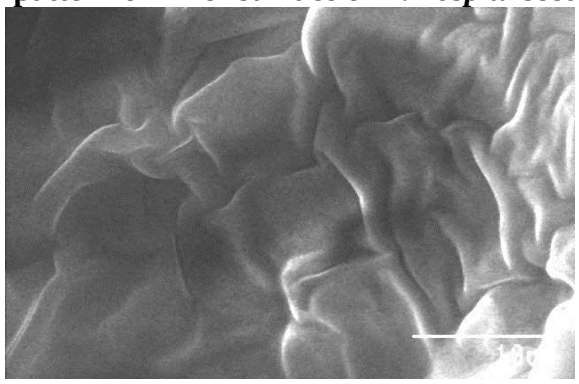

Fig. 11: SEM image of hydrocarbon isolated from crude extract of *K. hospita* flower.

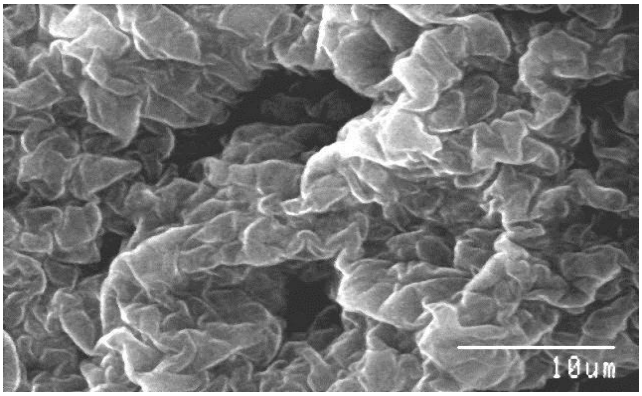

Fig. 12: SEM image of hydrocarbon isolated from crude extract of *K. hospita* seed.

CONCLUSION

The determination of n-alkanes has been characterised for the first time from leaves, flowers and seeds of *K. hospita*. SEM studies of different parts of epicuticular wax pattern and their hydrocarbons represent morphological tool for this plant. The variation of n-alkanes with higher odd: even ratio gets the idea about taxonomic marker. The presence of higher odd carbon in foliar waxes suggests an adaptive ability of the plant to grow in arid habitats and thus confirms the xerophytic nature of the plant.[15,16] Micro-morphological pattern deals with the idea of the deposition of n-alkanes in the surface of the plant parts. Thus n-alkane analysis and surface study give an idea about their topographical features and chemotaxonomic importance of the plant.

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