Indian Journal of Chemistry
Vol.36B, July 1997, pp.596 - 604

Humus, the epitome of Ayurvedio *makshika*

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Received 19 September 1996; accepted 10 March 1997

Ayurvedio *makshika*, a *maharasa* (rejuvenator, adaptogen), has been shown to be constituted of a large number of low M_r (mol. wt) humio intermediates, and medium and high M_r humio compounds. These results dispel a long standing misbelief that the bioactive ingredients of *makshika* constitute only inorganic minerals, viz. iron and chalco-pyrites. The stability of the *makshika*-humus core (str **5**) appears to be due to complexation with transition metal ions which produce resonance stabilised metallo-organic species (str **6a,b** and **7**). The low M_r organic compounds of *makshika*, in their natural habitats, find ecological niche within the micropores of humus and thereby fend off weathering and other extranuous onslaughts for ages. Humus seems to be not one but of all *maharasas*' epitome. The general features of *makshika* and shilajit are compared in the light of their origin and biological significance.

In Ayurveda, the term *maharasa* (function: rejuvenation, adaptogenic) denotes a group of drugs of mineral origin. Although there are eight accepted *maharasas*, *makshika* and *adrija* (meaning derived from rock, shilajit) are the only two drugs which have been mentioned in all the ancient texts of Ayurveda[1,2]. The other six, viz. *vaikranta* (manganite), *sasyak* (chalcenthite), *abhraka* (biotite), *vimala* (pyrite), *rasaka* (red blende) and *capala* (bismuthinite), have been mentioned only in a few Ayurvedic texts[1].

The varieties of *makshika*, viz. *rajata-* and *svarna-*, are known. According to the medical treatise, *Astangahrdaya* of Vagbhatta (AD 800-850)[2], the two varieties comprise iron and chalco pyrites, respectively. Iron pyrite, a sulphide mineral occurs worldwide in rocks of all ages and types. It is a common constituent of many ore veins, particularly those containing sulphides of Cu (chalco-pyrite), Ph, As, and Zn (ref. 3).

The occurrence of *makshika* has been reported from several states in India. It is abundantly available in many parts of Bihar and Himachal Pradesh as a sedimentary deposit. *Makshika* is an important subject of study for many reasons. Ayurveda has regarded it as a *maharasa* and grouped it along with *adrija* (=shilajit). The *sodhana* (purification) principles and therapeutic uses of both *makshika* and shilajit are, in many respects. similar[1,3]. But, while the chemistry of shilajit has been fully elucidated[3,4], practically nothing is known about the nature and chemical character of *makshika*. All that is known are of empirical nature. In contact with fire, *makshika* releases a viscous liquid which burns like bees-wax (hence the name). The presence of organic compounds in *makshika* is, therefore, likely. The occurrence of shilajit is restricted to sedimentary rocks[4]. In contrast, *makshika* occurs in rocks of all ages and types, as also in river bed and coal mine. The impact of these pedo- and geological differences of *makshika*, from those of shilajit, might be reflected on the chemistry and biological properties of the former. Pyrites that are associated with calcite, barite, and quartz, behave as 'somatoids' when foreign substances, e.g. colouring matter, plant and microbial debris are present during their formation and crystalliszation. Somatoids exhibit organized forms and reproducibility similar to those of lower forms of living beings. *Makshika* thus offers a unique opportunity to study its possible 'somatoid' manifestations. Finally, the study may find application in an improved formulation of *makshika* for use as an immuno-adjuvant (= *rasayan*).

Results and Discussion

Rajata- and *svarna-makshika* samples were collected from different places and sources of India and their identities were properly established. Each sample was extracted with organic solvents of graded polarity and the finger-prints of the extractives were monitored by HPTLC and HPLC

Table I—Low M_r organic compounds[a] of *makshika*[b]

Compound type	Identity of compounds (str nos) isolated from	
	Outer surface	Inner core[b]
Aliphatics		
hydrocarbons	C_{16}-C_{34} (n^- & branched)	C_9-C_{35} (n- & branched)
alcohols	C_{18}-C_{28}	C_8-C_{28}
aldehydes	—	n-heptanal to n-decanal[c]
Naphthenes		
cycloalkanes	—	dimethylcyclopentane[c], dimethylcyclohexane[c]
acids	—	methylcyclopentane carboxylic acid
Wax esters		
alcohols +	C_{22}-C_{28}	C_{14}-C_{28}
acids	C_{36}-C_{28}	C_{14}-C_{28}
Aromatics		
hydrocarbons	—	m- and p-xylenes[c]
acids	benzoic acid	benzoic acid, benzamide
phenolics	—	m-cresol[c], vanillin[c]
	vanillic acid, o-hydroxyacetophenone	vanillic acid, o-hydroxyacetophenone
Heterocyclics		
oxygen-	dibenzo-α-pyrones (**1-4**)	dibenzo-α-pyrones (**1-4**)
sulphur-	—	benzothiazole[c]

[a]Isolated by head-space collection, enfluenrage technique (see Experimental sec.)
[b]List of common compounds of *makshika* from different sources
[c]Volatile compound

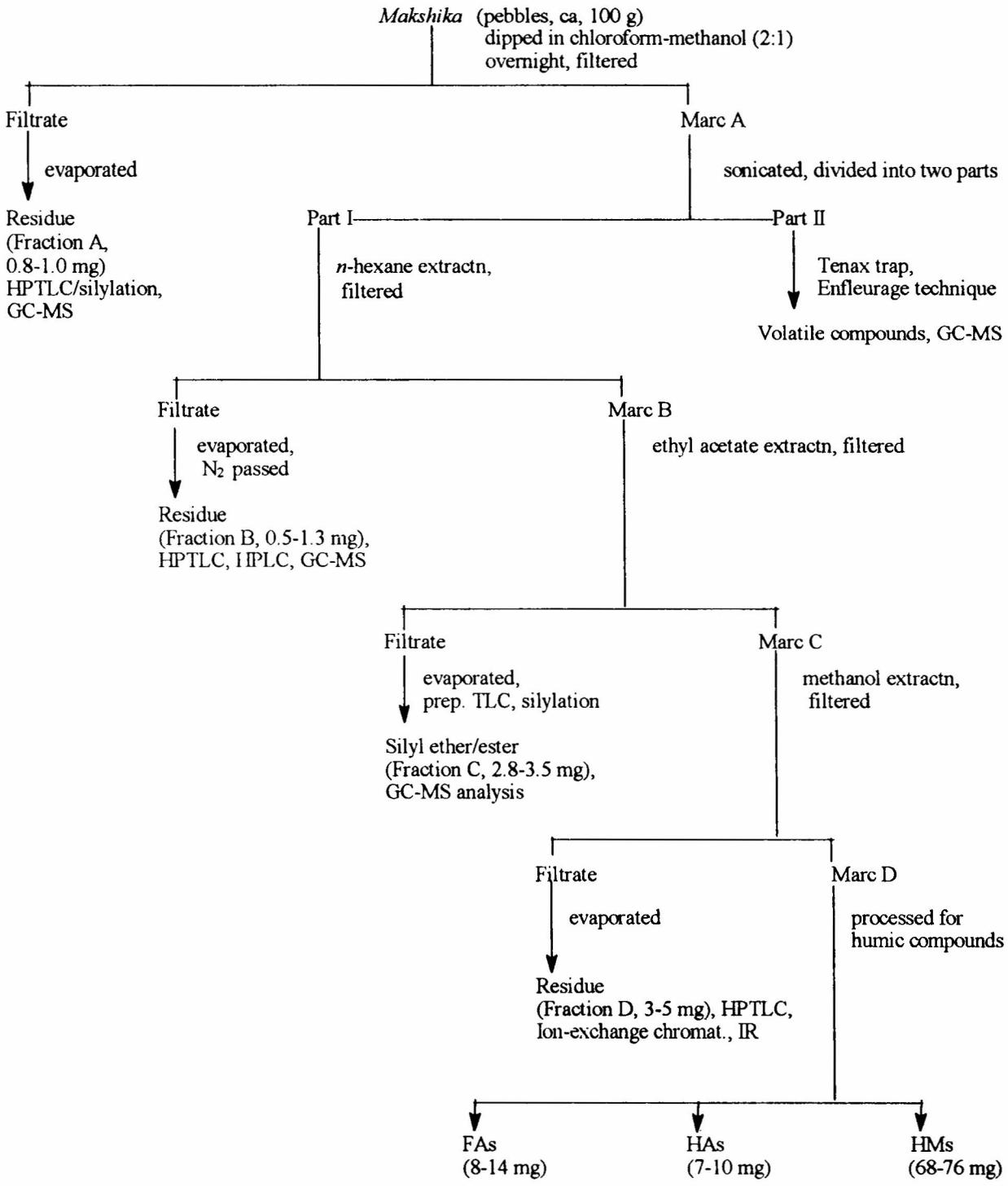
analyses. *Rajata-* and *svarna-makshika* extracts showed strikingly similar and complementary spectral HPTLC and HPLC patterns albeit of different intensities. The constituents from the outer surface and inner core of *makshika* were extracted and processed separately. Comprehensive chromatographic separation afforded several low M_r (mol wt) organic intermediates of humus[3,4]. The solvent-extracted *makshika* marc (SEM marc) was then processed for the three different types of humic substances according to a previously described procedure[3].

Humus-intermediates of makshika

The M_r humus-intermediates of *makshika*, unlike humus constituents, showed readily identifiable physical and chemical characteristics. These compounds were identified as aliphatic compounds, naphthenes (cycloalkanes), aromatic compounds (phenolic, carboxylic), and O- and S-heterocyclics (cf. Table I). Among these compounds, a number of volatile entities were encountered which remained trapped within the inner-core of *makshika*. These were isolated by use of a Tenax-type trap and by enfleurage technique[5]. The general mode of isolation and characterization of these compounds are depicted in Scheme I.

The prominent low M_r compounds included n-alkanes (C_9-C_{35}), the majority being in the C_{18}-C_{28} range with an even to odd carbon ratio of nearly 1. The distribution pattern of the alkanes and their even of odd carbon ratios suggested[6-8] the significant contribution of bacteria in the formation of the organic constituents of *makshika*. Since other low M_r aliphatic, aromatic and heterocyclic compounds, isolated from the inner core of *makshika*, , were seemed to be derived from the biotic (eco^-) and abiotic (geochemical/thermal) transformations of the high M_r molecules, e.g. proteins, carbohydrates and lipids. This postulate had procedent in the geochemistry of lipids[6].

Another important class of organic compounds, isolated form both outer surface and inner core of



Scheme I — Modes of isolation and characterization of organic compounds of *Makshika*

makshika, constituted oxygenated dibenzo-α-pyrones (str **1-4**, Figure 1). These compounds were earlier found in shilajit[4,9,10] Also, evidence was adduced to suggest that the oxygenated dibenzo-α-pyrones (and equivalents) constituted the 'central' building block (str **5**, dotted line substituted, Figure 1) of shilajit and of other terrestrial and aquatic humus[4,8,9]. The different degrees of structural complexities, e.g. allignment and chain-lengthening, due to eco^- and geological factors, were reflected in the composition of the humus-heteropolycondensates (M_r 700-100000).

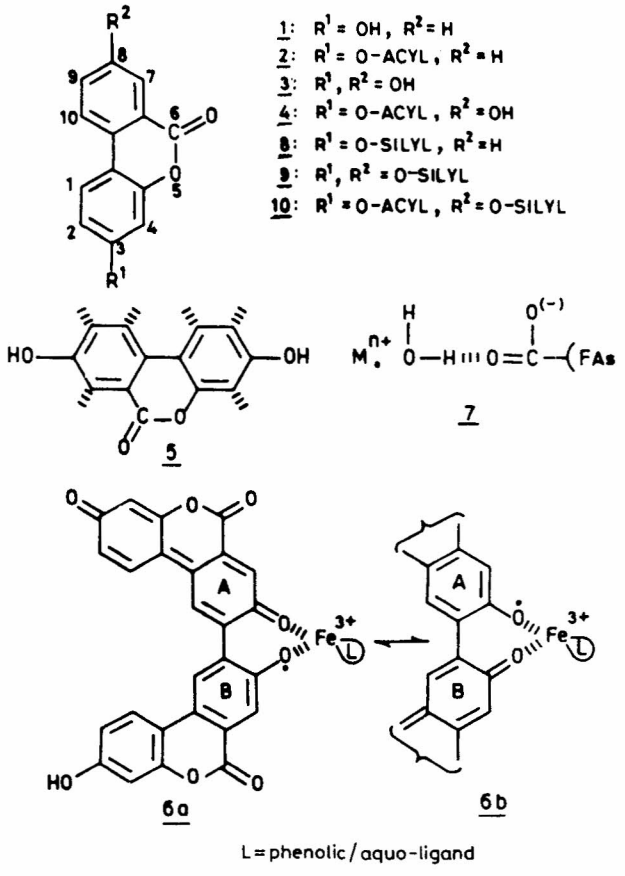


Figure 1 —Humic compounds of Ayurvedic *makshika*

Humus-heteropolycondensates of makshika

The *makshika* marc (SEM) was dissolved in aqueous alkali, under N_2 atmosphere, to obtain a mixture of fulvic acids (FAs), humic acids (HAs) and polymeric humins (HMs). The separation of these three types of humic substances was based on their solubility differences at different *p*H levels[7,11]. The FAs of *makshika*, like FAs from other terrestrial and aquatic sources[4,7,8], tenaciously retained some of low M_r organic compounds in their internal voids, e.g. by intercalation. These compounds were released by dissolving the *makshika*-FAs in aqueous alkali followed by extraction with ethyl acetate and *n*-butyl alcohol. These properties of *makshika*-FAs were reminiscent of shilajit-FAs which exist as spheroidal aggregates, perforated by voids (micropores) of variable diameters (100-500Å, as determined by X-ray crystallography and electron microscopy)[11]. The otherwise perishable organic compounds thus final ecological niche in these voids and thereby duck-away weathering (cosmic radiation, heat, oxidation) and other onslaughts (microbial infestation, air pollution) for ages.

Mild degradation of *makshika*-FAs by boiling with water, under pressure, yielded some additional low M_r compounds, e.g. *n*-, *iso*- and *ante-iso*-fatty acids, benzoic acid and phenolic acids, and vanillin. The *iso*-acids were mostly in the C_{14}-C_{18} range, and C_{15} and C_{17} acids were predominant amongst the ante-*iso*-acids. The occurrence of *iso*- and ante-*iso*-acids from *makshika* suggested the participation of mevalonate units (for head- and tail-condensation with poly-β-ketide units) in their biogenesis. The occurrence of branched chain hydrocarbons, pristane and phytane, of mevalonate origin, in oldest rocks, was considered as a strong circumstantial evidence for the contribution of microorganisms in the synthesis of organic compounds during a very early stage of the geology of earth[6]. As a corollary, the low M_r organic compounds of *makshika* Table I) would seem to be of very old origin.

ESR spectra of *makshika* and its organic fractions exhibited a single resonance line of 3 ± 2 gauss width (G) and the splitting factor (g) ranging from 2.0035 to 2.0045. These were characteristic of semiquinone free radicals derived from polyphenols[12]. The free radical concentrations of the different *makshika* samples ranged from 1×10^{11} to 3×10^{18} spins/gm. However, the spin concentrations of *makshika*-FAs were much lower, being in the range $1\text{-}2 \times 10^{11}$ spins/g. These and other analytical characteristics of *makshika* were compared with those of shilajit when a number of similarities between the two *rasayana* drugs were discerned (Table II).

The semiquinone free radicals of *makshika*-FAs were stabilized by chelation and complexation with the contained transition metal ions (Table II). One such donor-acceptor complex (str **6a, b**; L= *o*-hydroxyacetophenone, Fig. 1) was isolated from the *makshika* and its identity was established by direct comparison with the synthetic sample[13]. The grip on the metal ions in the complex (**6a, b**) was indicated by ion-exchange chromatography. Apart from the intra-molecular donor-acceptor complex of the type **6a,b**, metal ion-FAs ligand associations (e.g. layered) of widely differing stability are conceivable. By virtue of the presence of a large number of phenolic, carboxylic and carbonyl groups in the FAs molecules, in association with aquo-donor groups, complex formation of the type **7** (Fig. 1) is a distinct possibility. Such metal ion associations were decumented in shilajit humus[7,11].

That so many strikingly similar things could have originated in *makshika* and shilajit by chance is incredible. The common characteristics must have a bearing on their origin and metabolism.

Table II—Analytical profiles of *makshika* and shilajit humus

Parameter type	Humus source	
	Makshika	Shilajit
Organic compounds/Category	*Realtive abundance*[a]	
Low M_r compounds[b]	11.3	17.9
Fulvic acids (FAs)	12.8	21.4
Humic acids (HAs)	8.4	19.8
Humins (HMs)	67.5	40.9
E_4/E_6 values		
FAs	5.8 ± 1.2	8.8 ± 0.8
HAs	3.2 ± 0.33	4.1 ± 0.22
Metal ions (as FAs-complexes):		
Fe	0.1-1.5	0.1-0.8
Cu	0.002-0.005	traces
Ca	0.1-0.4	1.2-2.0
ESR data		
g value	2.004 ± 0.0005	2.0025 ± 0.001
Spins/g	1×10^{11}-3×10^{18}	1×10^{14}-2.5×10^{16}
Number average *mol wt* ($\bar{M}n$)[c]		
FAs	680-975	750-2255

[a]Mean of three replicates

[b]Combined organic volatiles and solvent extractives (see Table I, Scheme I)

[c]By vapour pressure osmometry[9]

Both were derived essentially from the weathering of rocks (humification) involving rhizospheric microflora. However, one significant difference between the two constitutes the diversity of their natural habitats. While shilajit originates in and remains stationary in sedimentary rocks[4], *makshika* occurs in rocks of all ages and types and is often transported to far distant places. The impact of these differences were reflected in the extent, inter alia, of concentrations of semiquinone free radicals and in the degree of heterogeneity of the respective humic substances. As expected, *makshika*-FAs exhibited smaller $\bar{M}n$ (number average mol. wt) and E_4/E_6 values compared to those of shilajit-FAs (Table II). These indicated longer residence period of *makshika* in its natural habitat. In contrast, *makshika* itself showed larger range and higher concentrations of semiquinone free radicals and higher abundance of polymeric humins (HMs) compared to those of shilajit (Table II). These properties suggested extended period of metabolism and hence of older origin of *makshika*.

Biological potential

The reducing property of FAs (through the polyphenol moieties) would cause $Fe^{3+} \rightarrow Fe^{2+}$ reduction systemically resulting in mobilization and functionalization (e.g. attachment to haemoglobin) of systemic iron. This would occur not only under anaerobic condition but also in the presence of oxygen and the attendant reactive oxygen species (ROS). The biological implication of this phenomenon could be pronounced[14,15]. Cellular protection against deleterious effects of ROS, generated in aerobic metabolism, in the presence of loose Fe^{3+} (Haber-Weiss reaction)[16] is organized at multiple levels. Defence strategies include capturing of loose iron ions, by way of $Fe^{3+/2+}$... complex e.g. **6a, b** and **7**, formation. The complex, in turn, would augment cell viability and function[17,18]

The metallo-FAs complexes are known to contribute significantly to plant physiology[7,11]. The hydrated metal ion complexes of the type **7**, being soluble in both hydrophobic and hydrophilic solvents, would be more available for the biochemistry of soil organisms[18]

Conclusion

Makshika, an Ayurvedic *maharasa*, has been analysed for the first time by comprehensive chemical, chromatographic and spectroscopic methods. This has been done with the intension also of de-

termining its relation with shilajit, another *maharasa*. The general features of *makshika* and shilajit are similar in many respects (Tables I and II). Both are resulted by the process of humification on rocks, where bacteriamineral interaction was the main contributing factor. However, while shilajit is originated and restricted only to sedimentary rocks, *makshika* is derived in rocks of all ages and types and is often transported to far distant places from its natural habitat. These pedo- and geological differences between shilajit and *makshika* are reflected in their chemistry and metabolism. When the source of raw materials for humifications is cut off or depleted, humus then is exposed exclusively to metabolism (biotic/abiotic) resulting in low M_r polycarboxylated FAs (small $\bar{M}n$) and intractable polymeric humins (HMs). Both these things happended in *makshika*. In contrast, when humus is continuously produced, as it is degraded, a mixture of fresh and paleo-humus (remnants) would ensure, e.g. in shilajit. But one aspect is strikingly common to both. Organic chemicals that find ecological niche in the micropores of humus are preserved for a longer period of time in both *makshika* and shilajit. Also, the presence of iron ions in both *makshika* and shilajit inhibit mineralization of their humus. However, the large amounts of toxic polymeric quinones (HMs) and comparatively smaller amounts of therapeutically active ingredients in *makshika* would warrant greater care for their processing and standardization.

The study dispels a long-standing misbelief that the bioactive ingredients of *makshika* constitute only inorganic minerals. In fact, initial results would seem to indicate humus to be the epitome of all Ayurvedic *maharasas*. Work in this direction is currently in progress in our laboratories.

Experimental Section

Test compound. Rajata- and *svarna-makshika* samples were collected from Santhal Parganas and Hazaribagh districts in Bihar and from Rampur in Himachal Pradesh, and were properly identified. The specimens have been preserved in the library of Prof. S Ghosal for future reference. The *makshika* samples were also obtained from market and from the Pyrites and Phosphates Chemical Limited (PPCL), New Delhi.

The following techniques and methods were applied for the isolation and characterisation of the organic constituents of *makshika*.

Column chromatography. Silicic acid (Mallinckdrodt, 100 mesh) was used as the adsorbent; solvents of graded polarity, *n*-hexane, hexane-diethyl ether (95.5:0.5, 95:5, 90:10) and diethyl ether, were used as eluents. The eluates were monitored by HPTLC and similar fractions were combined for further processing.

HPTLC. CAMAG TLC (plate material, Silica gel 60 F_{254}) evaluation assembly (CATS 3.16/ Scanner II V 3.14) was empolyed. The detection was done by both fluorescence and quenching mode. Two solvent systems, viz. chloroform-methanol (90:10) and *n*-butyl alcohol-acetic acid-water (4:1:2), were used as the developers.

HPLC. Waters Associate HPLC assembly with a RP-8 reverse phase column, equipped with both PDA and RI detectors, was empoyed. Methanol-water (80:20) and acetonitrile were used as eluants.

GC-MS. Gas chromatographic separation was performed on a OV-1 (30 m × 0.33 mm) capillary column; oven temperature was programmed from 80°C (1 min hold) to 320°C (15 min hold) at 4°C/ min-rise; injection temperature was 250°C. MS was obtained on a Hitachi M-4100 instrument, at an ionization potential of 70 eV.

Electron probe microanalyser (EPMA). The conditions were ACC. V (kV) 20.0; S.C (micro-) 0.20, beam size, 30-150 μm.

ESR. Electron spin resonance spectra of the *makshika*-humus were recorded on a Varian E-112 spectrometer, according to literative procedure[12]. Briefly, dired and finely powdered samples of *makshika* and of the corresponding humic compounds, FAs, HAs and MHs, were taken separately in standard ESR tubes. The spectra were recorded at room temperature using 100 KHz modulation and a microwave frequency of 9.3 GHz.

E_4/E_6 ratio. The absorbance ratios at λ 465/ 665 nm of the humic substances were determined in a Beckmann spectrophotometer. HAs was dissolved in aqueous $NaHCO_3$ (50 mM) solution. FAs being soluble at all *p*H values (in the range 1-9), its E_4/E_6 ratios were determined at different *p*H; the effect of *p*H on the E_4/E_6 values of *makshika*-FAs was marginal.

Isolation of organic constituents of makshika

The general method of isolation and mode of characterization of the organic constituents of *makshika* are depicted in the Scheme I. The HPTLC finger-prints of the *makshika* constituents from different collections were almost superimposable albeit of different intensities. *Rajata-makshika* collected from Bihar showed highest yields of organic constituents and was used for detail analyses (Scheme I). Column chromatography of *makshika* extractives was conducted for large scale isolation

of lipids and oxygenated dibenzo-α-pyrones (str **1-4**, Fig. 1).

Head-space collection and analysis of makshika. Makshika (pebbles) sonicated at 30°C for 6 hr, was placed in a two-arm flask, equipped with a Tenax-trap[5] on one arm; and through the other arm, pure N_2 was passed at ordinary temperature. The volatile constituents of *makshika* were slowly accumulated on the trap. These constituents were subsequently desorbed into a GC-MS analyzer. Several head space attempts were made to obtain detectable quantities of the volatile constituents from the inner core of *makshika.*

Enfleurage technique. In another set of experiment, the volatile constituents of pulverised *makshika* (10-12 g) were adsorbed into a fresh fat mixture (triolein-tripalmitate in 1:10 ratio). After about 16 hr, the adsorbed constituents were extracted with acetonitrile. The extractives (lyophilized) from the acetonitrile solution were subjected to HPLC (acetonitrile as the eluant) and GC-MS analysis.

GC-MS analysis. The volatile constituents of *makshika* were analysed as follows. The peaks from scan nos. 40 to 160 were expanded and all the high intensity peaks from mass numbers m/z 128 to 492 were monitored. The presence of alkanes containing C_9 to C_{35} compounds was established by using reference samples. Likewise, scan nos. 180-330 showed the presence of alkanals: $C_7H_{14}O$ (m/z 114, M^+), $C_8H_{16}O$ (m/z 128), $C_9H_{18}O$ (m/z 142), $C_{10}H_{20}O$ (m/z 156), exhibiting appropriate fragmentation patterns in each case; scan nos. 345-412 were due to naphthenes, dimethylcyclopentane, C_7H_{14} (m/z 98, M^+, prominent fragment ion peaks at 97, 71, 70) dimethylcyclohexane, C_8H_{16} (m/z 112, M^+, fragment ion peaks at 111, 84, 43, 42), methylcyclopentane carboxylic acid, $C_7H_{12}O_2$ (m/z 128, M^+, 111, 110, 73, 44); scan nos. 428-467 due to *m*- and *p*-xylenes, C_8H_{10} (m/z 106, M^+, 78, 77), scan nos. 560-581 due to benzoic acid, $C_7H_8O_2$ (m/z 122, M^+, 105, 77) and benzamide, C_7H_7NO (m/z 121, M^+, 103, 102, 77), scan nos. 732-745 due to benzothiazole, C_7H_5NS (m/z 135, M^+, 134, 103, 102, 101, 77) and a higher homologue, C_8H_7NS (m/z 149, M^+), and scan nos. 780-810 due to vanillin, $C_8H_8O_3$ m/z 152, M^+, 137, 133, 132, 109) and *o* hydroxyacetophenone, $C_8H_8O_2$ (m/z 136, M^+, 121, 94, 77) (Table I). The molecular formula of each compound was established by high resolution mass spectrometry.

Solvent extraction of makshika. In a typical experiment (see Scheme I), *makshika* pebbles (*ca* 100 g) were dipped in Folch's solvent ($CHCl_3$-MeOH, 2:1, 100 mL) at room temperature, overnight. The extract was filtered and the filtrate was lyophilized. A part of the extractives (0.5 mg) was redissolved in $CHCl_3$-MeOH (0.5 mL) and subjected to HPTLC and HPLC analyses. The results showing organic constituents of the outer surface of *makshika* are given in Table I.

Fraction A (*Scheme I*). HPTLC and HPLC analyses of this fraction showed the presence of benzoic acid and *o*-hydroxyacetophenone as major entities. Additionally, the oxygenated dibenzo-α-pyrones (str **1-4**, Fig. 1) were detected in traces.

The corresponding marc was sonicated divided into two parts. From one part, the volatile constituents were traped and analysed (*supra*). The other part was extracted successively with *n*-hexane, ethyl acetate and methanol to give fractions B-D, respectively.

Fraction-B. HPTLC ($CHCl_3$-MeOH, 90:10) and HPLC (MeOH-H_2O, 80:20) comparison of this fraction with the wax esters of shilajit[5] showed strong similarities. The mixture was silylated before and after acid (HCl) hydrolysis for analysis of polar constituents.

Silylation. The mixture of organic compounds (ex-Fraction B) (*ca* 0.5 mg) was dissolved in $CHCl_3$-MeOH (2:1, 10 μL), to which N,O-bis(trimethylsilyl)-trifluoroacetonitrile (Wako, Japan) (5 μL) was added. The mixture was kept at 60°C for 1 hr and then subjected to GC-MS analysis.

In GC-MS analysis, scan nos 608-654 showed the presence of silyl esters of C_{16}-C_{28} fatty acids (*n*-, *iso*- and ante-*iso*), vanillic acid (as silyl ether-ester, m/z 312 (M^+), 297, 267, 258, 223, 193, 126, 73); and scan nos 838-926 showed the presence of 3-P-silyldibenzo-α-pyrone (str **8**) (m/z 284 (M^+), 212, 211, 184, 154, 153, 73, 72), 3,8-di-O-silyldibenzo-α-pyrone (**9**) (m/z 372 (M^+), 299, 227, 226, 200, 172, 171, 73), 3-O-stearoyl/palmitoyl-dibenzo-α-pyrone (str **2**) (m/z 478/450 (M^+), 267, 266, 239, 238, 229, 212, 211, 184, 153), 3-O-stearoyl/palmitoyl-8-O-silyldibenzo-α-pyrone (**10**) (m/z 566/538 (M^+), 267, 266, 239, 238, 229, 226, 200, 172, 73) and 3-O-stearoyl/palmitoyl-8-hydroxydibenzo-α-pyrone (**4**) (m/z 494/466 (M^+), 266, 239, 228, 227, 200, 199, 173, 172, 150).

Fraction C. A portion of this fraction was subjected to prep. TLC followed by HPTLC and HPLC analyses using markers when the presence of compounds **1-4, 9** and **10** was established. The remaining portion was silylated as described above

and then subjected to GC-MS analyses to support the above identification.

Fraction D. The major constituents of this fraction were in complex association with metal ions (Table II). One of these constituents was separated by prep. TLC and by paper electrophoresis[13]. Its identity was established as 6*a*, *b* by direct comparison (HPTLC), superimposable IR spectra) with a synthetic sample[12,13].

Column chromatography of the fractions C and D afforded the dibenzo-α-pyrones (**1-4** and **6a,b**) in quantities sufficient for their complete characterization and immunological evaluation *in vitro*.

Ion-exchange chromatography. Fraction D FAs (vide *infra*) (in 10-100 mg portions) were dissolved in distilled water (10 mL). To each flask, 1*N* KCl (0.5 mL) was added. Dowex resin (50W×8; 1 g) was separately saturated with K^+ ion. The solutions of fraction D or FAs were transferred to K^+-saturated Dowex resin in Erlenmeyer flasks. Each mixture was shaken at 25±2°C for 1 hr. The exchange resin was then removed by filtration. The filtrate and washings, containing metallo-organic complexes of *makshika*, were combined and evaporated. The residue was analysed by EPMAS and the results are given in Table II. The free phenolic constituents present in the residue were converted into silyl derivatives as described above and analysed by GC-MS. Only traces of the oxygenated dibenzo-α-pyrones (str **1** and **3**) were detected as their silyl ether derivatives. The residue from the ion-exchanged fraction D was boiled with aq. HCl, the acid was removed, and the product was subjected to HPTLC (*n*-butyl alcohol-acetic acid-water, 4:1:2). A large number of freshly-released dibenzo-α-pyrones, including **1** and **3**, and oligomers were detected by using markers.

Humic substances. The powdered marc (methanol-insoluble residue, Schemer I) was triturated with an aqueous solution of alkali (0.1*N* NaOH); N_2 was passed through the mixture for **1** hr to remove the dissolved gaseous entities and to prevent oxidation of phenolic compounds during the alkali treatment. The solution was filtered and the filtrate acidified with HCl to *p*H ~3. The acidified solution was kept at ambient temperature overnight. The mixture of precipitated humins (HMs) and humic acids (HAs) were collected by centrifugation. The acidic supernatant was extracted with ethyl acetate and *n*-butyl alcohol to isolate the freshly released low M_r organic compounds from the micropores of fulvic acids (FAs). The aqueous acidic mother liquor was then adsorbed on activated charcoal (10 g). The adsorbed FAs were eluted from the charcoal by treatment with acetone (100 mL). The yellowish-brown acetone solution was evaporated and desicated to give FAs as a light-brown powder (Table I). The FAs did not melt up to 360°C; IR (KBr): 3400 (broad, bonded OH), 2926 (aliphatio CH_3), 1780-1700 (br, CO), 1650 (br, C=C), 1020-1000 cm^{-1} (OH); EI MS (major peaks): m/z 466, 452, 438, 424, 410 (due to M^+ of C_{31}-C_{27} fatty acids in which odd carbon compounds exhibited higher abundance); 228, 227 (due to 3-O-acylated 3,8-dioxygenated dibenzo-α-pyrones); 212, 211 (3-O-acylated 3-oxygenated dibenzo-α-pyrones); ^1H NMR ($CDCl_3$-DMSO-d_6; 100 MHz) δ 8.3-6.2 complex H, integration not accurate, aromatic/cojugated olefinic protons), 2.2 (fatty acyl $-CH_2-$), 1.2-0.8 (aliphatic $-CH_2-$ and CH_3). These data indicated the contribution of both phenolics and lipids in the constitution of the *makshika*-FAs.

The *makshika*-HAs were separated from the HMs-HAs mixture by dissolving it in a dilute aqueous solution of $NaHCO_3$ in which HMs were insoluble and filtered off. The aqueous alkaline filtrate on acidification afforded HAs.

Hydrolysis of makshika-FAs. Makshika-FAs (11 mg), after extraction of the non-humic organic constituents from the micropores, were hydrolyzed in water at 100°C in a pyrex sealed tube for 6 hr. The mixture was cooled, the solvent evaporated, and the residue desicated. It was dissolved in $CHCl_3$-MeOH (2:1) and subjected to HPTLC, HPLC and GC-MS analyses.

Acknowledgement

We thank Dr K Kawanishi, Kobe Pharmaceutical University, Japan, for some of the crucial spectral determination; Mr Brijpal, R&D, Indian Herbs, Saharanpur, for skillful technical assistance, and Messrs Indian Herbs, Saharanpur, for sponsoring the project.

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