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Research Article

MORUS ALBA L., A NEW PERSPECTIVE: SCANNING ELECTRON MICROSCOPIC, MICRO CHEMICAL, GC-MS AND UPLC-MS CHARACTERISATION

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ABSTRACT

Present investigation was aimed at studying the micro morphology and elemental analysis of *Morus alba L.* Fresh plant parts were fixed in Glutaraldehyde, dehydrated and observed in Scanning Electron Microscopy (SEM) and carried out Energy Dispersive X -ray analysis (EDAX). Scanning electron microscopic studies revealed the characteristic features of the plant. EDAX studies revealed the plant's enrichment with calcium and magnesium and potassium enrichment and presence of aluminum in fruits. Dried leaves were sequentially extracted in hexane, chloroform and methanol. Hexane and chloroform extracts were subjected to GC-MS. Methanolic extract was analysed by UPLC-HRMS. Details of the chemical constituents and their medical significance is discussed.

Keywords: Morus alba, Scanning Electron Microscopy, EDAX, Micro chemical Analysis, GC-MS.

INTRODUCTION

Morus alba (Mulberry) is known for its medicinal value. Its medicinal values include antihelmenthic, odontalgic, expectorant, hypoglycemic, emetic and also used as a cure for dysentery and as laxative. Chinese medicine recognized the medicinal value of *M. alba,* to treat fever, for liver protection, joint strength as antihypertensive and diuretic[1]. Japanese consume *M. alba* leaves as tea. In Korea and Japan mulberry is used as antihyperglycemic food[2]. Mulberry leaves are rich in flavonoids[3,4]. Mineral composition of mulberry fruits employing atomic absorption Spectrometry was reported earlier[5]. Few prenylflavanes, a glycoside and benzyl-glucopyranoside were isolated from mulberry leaves and reported their hypocholesteromic activity and the prevention of atherosclerosis[6]. Hypotriglyceridemic effect of *M. alba* was also reported earlier[7]. *M. alba* is mostly cultivated for its leaves to feed silk worms in China and for fruits in Europe.

Though mulberry is very popular for its varied medicinal uses, reports are lacking on its morphological characteristics and elemental (mineral) composition. Many pharmacopeias have recognized the micro morphological characterization as an essential part of pharmacognostic evaluation of plant drugs[8-10]. Both light and Scanning Electron Microscopes (SEM) are handy for this purpose. SEM has an added advantage in direct observation of thick samples[11-14]. Hence an attempt was made to elucidate the morphological characteristics of *M. alba* employing SEM.

In Ayurvedic and many other traditional medicinal systems, trace elements are described as "inorganic switches"[15-17] . Therapeutic relevance of mineral components of various medicinal plants was well evaluated [18-22]. Various techniques are available for the quantification of minerals, viz., AAS, EDXRF, ETAAS, ICPAES, ICPMS and EDAX/EDX/EDS. The later is versatile, non destructive and facilitates quick multiple sampling even with a very low quantity of

sample[22-24, 13, 14]. EDAX fitted to SEM was chosen for evaluating mineral composition.
Hyphenated techniques like GC-MS, LC-MS and UPLC-HRMS facilitate rapid separation and identification of components of the plant extracts. Reports in this regard are voluminous[25-35]. Most of the studies were based on extracts of a single solvent. In the current investigation, leaves of *M. alba* were sequentially extracted with hexane, chloroform and methanol. Hexane and chloroform extracts were analysed in GC-MS. UPLC_HRMS studies were carried out on methane extract.

MATERIALS AND METHODS

Leaves and various parts of *M. alba* were collected from IICT campus and washed thoroughly with water. Fresh samples were processed for SEM & EDAX as described by Yashvanth et al., 2010, 2012. Leaves were air dried in shade till getting consistent dry wt. Dried leaves (500g) were extracted with hexane, chloroform and Methane sequentially in soxhlet apparatus and vacuum dried. Phytochemical studied were carried out using GC-MS and UPLC-HRMS.

GC-MS studies

Hexane and chloroform extracts were analysed using Agilent GC/MS, Model 6890 NGC with 5973 inert MSD with the analytical conditions – ZB-5-MS column (30ml, 0.25mm ID, 0.25µm film) filled with Dimethyl poly siloxane; 1ml of sample quantity; Program gradient temperature of the oven with 50ºC for 2m with an increment of 10ºC to 280ºC with a holding time of 27m; injector temp. 250ºC; ion source temp. 230ºC, EI (70eVIE) with a scan interval of 0.01S. Wiley, 2007 library was used
for comparison of spectra and identification of the compounds.

UPLC-HRMS studies

The analysis was carried out on a Thermo Exactive ™, (Thermo Scientific) Orbitrap mass spectrometer coupled to Accela 600 (Thermo Fisher Scientific Inc) ultra-performance liquid chromatography (UPLC) pump and an Accela auto sampler. The separation was carried out on Hypersil gold C_{18} column of length 50 mm, 2.1 mm internal diameter, and 1.9 µm particle size (Thermo Scientific). The column was operated at ambient temperature with a Security Guard™ Ultra C_{18} guard column with 2.1mm internal diameter (Phenomenex). The mobile phases comprise of water (A) and Acetonitrile (B), both with 0.1% formic acid to maintain uniformity. The separation was carried out in gradient mode starting from 0% B increased up to 98% till 25 min, then increased to 100 %A from 25.11 min up to 30 min. ESI positive mode was employed for obtaining mass spectra. Compounds were identified by comparing the mass values with the existing databases[36, 37].

RESULTS

Scanning Electron Microscopy

Stem surface was covered with two types of hairs – 1. slender, elongated and whip like measuring >350 microns and 2. stout, curved measuring >30 microns. Sessile glands, of spherical and poly hedral tips (arrow) were aplenty along with exudates and crystals (block arrow) on the stem surface (figure 1A). In transverse section, stem was quadrangular and there is no clear cut demarcation of the lobes. Epidermis was single layered with suberised sub spherical cells. Sessile glands with enlarged basal cells were embedded in the epidermis and protruding into the cortex. Cortex was parenchymatous, 8 to 9 layers with thick cell walls and irregular contours. Cambium was distinct and multilayered, with phloem towards the exterior and endarch xylem towards the center. Xylem vessels present in the four corners were larger than those present in the middle and thick patches of Sclerenchyma were observed surrounding them towards the periphery. Medulla was parenchymatous with irregular cells, filled with phenolic contents (figure 1B).
Figure 1C depicts the cross section of leaf blade. Upper and lower epidermis was distinct and single layered. Upper epidermis was thick. Compact single layered palisade tissue was noted below the upper epidermis. This was followed by compact parenchymatous tissue. Club shaped multi celled rudimentary hairs (arrow) and sessile glands with spherical base and tapered tip (block arrow) were scene on the lower surface of the leaf (figure 1D). Stomata were paracytic (figure 1E). Upper leaf epidermis was covered with wax and stomata were not visible. Sessile glands were noted. They differ in their morphology with those found on the lower leaf surface; they were spherical and the tapering tips were absent. Exudates were seen getting secreted out from few glands (arrow, figure 1F).
Surface of the calyx was covered with ribbon like, single celled, undulated hairs of < 250 microns. Both the types of sessile glands described ahead were present on the surface of the calyx (figure 1G). Surface morphology of corolla was similar to that of the calyx. Stamen was having small stalks, bi lobed with semi

spherical curvature. Each lobe showed further incomplete bifurcation. Pollen was polyhedral in shape with distinct undulated surface pattern (figure 1H).

Micro Chemical (EDAX) Analysis

Carbon, Oxygen, Silica, Sodium and magnesium were detected in all the parts studied. There was a variation in their percentages. Carbon & Oxygen ranges were 46.11-82.72 and 13.77-51.32 respectively. Highest carbon content was found in crystal on leaf surface and the lowest was in fruit. Highest oxygen content was in calyx and the lowest was in crystal on the leaf surface. Sodium and Magnesium ranges were 0.12-0.45, 0.01-0.80 respectively. Lowest sodium was in calyx while the highest was in corolla. Aluminum was detected in crystal on leaf surface and in fruit. Chlorine was restricted to fruit surface. Potassium was detected in the fruit (Table 1&2).

GC-MS Analysis

GC-MS analysis of hexane and chloroform extracts showed 9 and 6 compounds respectively (Table 3). Majority of the detected compounds were hydrocarbons and fatty acids ; loliolide from Chloroform extract is a terpenoid.

UPLC-HRMS Analysis

Thirty seven compounds were resolved and identified from the methanolic extracts of the leaves. Of them 29 compounds were previously reported from *M. alba* ; eight of them were reported from other species of the genus *Morus* (Tables 4, 5). Detected compounds include alkaloids, 2-aryl benzofuron derivatives, flavone/ coumarin glucosides, flavone derivatives, amino acids, phenols and phenol derivatives.

DISCUSSION

Since long microscopy gained importance in evaluation of plant drugs[11-13,8-10,38,39]. Reports on the use of Electron Microscopy for pharmacognostic evaluation are sparse and there is a need for enhanced use of electron microscopy in the characterization of plant drugs. Micro morphological features like pattern of hair on the stem, quadrangular outline of the stem cross section, schlerenchymatous patches in the four corners of the stem, compact leaf tissue, glandular pattern, club shaped, multi cellular, rudimentary hairs on leaves and floral parts, pollen shape and pattern on the pollen wall exhibited by *M. alba* are distinct and facilitate easy identification of the plant. SEM played significant role in elucidating these characteristic features.

EDAX studies revealed the enrichment of calcium and magnesium in vegetative parts of the plant; fruits are rich in potassium; aluminum was also found on fruit surface. Presence of Aluminum and the richness of potassium in the mulberry fruits were reported earlier[40, 5]. These elements might play their role in the therapeutic action of the plant. Variation in the elemental composition of different plant parts and phylogenic variation in several species was reported previously[38. 39]. Significance of various elements with respect to human physiology was well discussed[41].

Of the three solvents used for extraction, methanolic extract showed higher yield. Similar observation was reported earlier[42]. It was also reported that methanolic extract showed stronger antioxidant activity. Phytochemical constituents detected fall under varied chemical groups viz., alkaloids, glucosides, flavonoids and amino acids that have potential medicinal uses. Deoxynojirimycin was known to inhibit glycogenlysis, act as anti viral (HIV), anti hyperglycemic, purgative, vermifuge and a potential curative agent for cancer and obesity[43, 44]. Antibacterial activity was reported for Kuwanon G[45]. Mulberrofuran G was found to have curative action against Hepatitis B[46]. Mulberry Moracins were descried as scavengers of UV stress-generated free radicals[47]. Morusimic acid is a pyrrolidine amino acid with antidopamenergic activity. Anti-dopominergic effect of methanolic extract of mulberry leaves was previously reported[48].

CONCLUSIONS

It can be concluded that the current investigation is a new perspective on *M. alba*, since it revealed several diagnostic features of the plant, that are of use to authenticate and standardize the plant drug. It also explored the chemical constituents from extracts of three different solvents viz., hexane, chloroform and methanol.

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Table 1: Elemental composition (weight & Atomic weight* %) of Stem & Leaf of *M. alba*

S. No.	Element	Stem Surface	Stem Cross section	Leaf Upper Surface	Leaf Lower Surface	Leaf Cross Section	Crystal on leaf
1	Carbon	64.14 (70.85)	67.23 (73.90)	50.83 (58.60)	48.45 (56.11)	77.82 (82.56)	82.72 (87.62)
2	Oxygen	34.34 (24.48)	30.47 (25.14)	46.72 (40.43)	49.30 (42.89)	22.53 (17.15)	13.77 (10.95)
3	Aluminum	-	-	-	-	-	0.27 (0.13)
4	Silica	1.05 (0.05)	0.92 (0.43)	0.45 (0.22)	0.72 (0.35)	0.33 (0.16)	1.11 (0.50)
5	Chlorine	-	-	-	-	-	0.41 (0.15)
6	Calcium	0.38 (0.13)	1.09 (0.36)	1.78 (0.62)	1.2 (0.42)	0.13 (0.04)	1.24 (0.39)
7	Sodium	0.03 (0.02)	0.18 (0.10)	0.02 (0.01)	0.14 (0.09)	0.17 (0.10)	0.43 (0.24)
8	Magnesium	0.06 (0.03)	0.11 (0.06)	0.19 (0.11)	0.19 (0.11)	0.01	0.05 (0.03)

* values in parenthesis

Table 2: Elemental composition (weight & Atomic weight* %) of various parts of *M. alba*

S. No.	Element	Calyx	Corolla	Anther	Fruit	Fruit Section
1	Carbon	47.09 (54.06)	54.85 (62.88)	58.90 (65.82)	46.11 (54.77)	46.11 (56.29)
2	Oxygen	51.32 (44.67)	41.06 (35.34)	40.33 (33.83)	48.37 (43.13)	39.56 (36.24)
3	Aluminum	-	-	-	0.13 (0.07)	10.82 (5.88)
4	Silica	0.63 (0.31)	0.52 (0.25)	0.28 (0.14)	0.45 (0.23)	1.43 (0.75)
5	Chlorine	-	-	-	0.14 (0.06)	0.05 (0.02)
6	Potassium	-	-	-	4.35 (1.59)	-
8	Sodium	0.12 (0.07)	0.45 (0.27)	0.21 (0.12)	-	0.33 (0.21)
9	Magnesium	0.23 (0.13)	0.80 (0.45).	-	-	0.01

*values in parenthesis

Table 3: Chemical composition of hexane and chloroform extracts of leaves of *M. alba*

Hexane Extract		Chloroform extract	
Retention time (m)	**Compound/Molecular formula/Molecular Wt.**	**Retention time (m)**	**Compound/Molecular formula/Molecular Wt.**
10.573	Dodecane / $C_{12}H_{26}$/ 170.33	11.438	Loliolide (2(4H) Benzofuranone) / $C_{11}H_{16}O_3$ / 196.24
13.353	Tetradecane / $C_{20}H_{38}$/ 278.52		
15.824	1. Hexadecane / $C_{14}H_{30}$ / 198.39		Neophytadiene / $C_{20}H_{38}$/ 278.52
18.061	Octadecane / $C_{18}H_{38}$/ 254.49	11.611	Hexadecanoic acid methyl ester
18.555	2-Pentadecanone, 6,10, 14 trimethyl- / $C_{18}H_{36}O$ / 268.47	12.056	2. Hexadecanoic acid / $C_{16}H_{32}O_2$ / 256.42
19.383	Hexadecanoic acid / $C_{16}H_{32}O_2$ / 256.42	12.254	
20.087	Eicosane / $C_{20}H_{42}$/ 282.55	12.933	Octadecatrienoic acid methyl ester / $C_{19}H_{32}O_2$ / 292.45
21.224	Phytol / $C_{20}H_{40}O$ / 296.54	13.143	Octadecatrienal / $C_{18}H_{30}O$ / 262.43
29.057	Nonodecene / $C_{19}H_{40}$ / 268.51		

Table 4: Chemical composition of methanol extract of leaves of *M. alba*

Retention time (m)	Compound	Molecular formula / ion mass
3.70-3.83	L-(+)-Tartaric acid	$C_4H_6O_6$ / 151.04
4.19-4.29	3,4',5,6-Tetrahydroxy flavanone	$C_{15}H_{12}O_6$ / 290.85
4.82-4.97	Skimmin	$C_{15}H_{16}O_8$ / 325.11**
5.13-5.19	Thiamine	$C_{12}H_{17}N_4OS$ / 266.12**
5.58-5.69	Moracin D	$C_{19}H_{16}O_4$ / 308.13
9.00-9.13	Benzyl- β-D-glucopyranoside	$C_{13}H_{18}O_6$ / 294.15*
9.61-9.86	Moracin P	$C_{19}H_{18}O_5$/ 328.14
10.85-11.03	Malberroside A	$C_{26}H_{32}O_{14}$ / 568.30
(24.85-25.03)		
11.19-11.38	1-Deoxynojirimycin	$C_6H_{13}NO_4$ / 163.04**
11.63-11.80	Moracin N/R	$C_{19}H_{20}O_6$ / 346.26**
(18.25-18.36)		
11.96-12.12	4-Hydroxy-3,5-dimethoxy benzyl alcohol	$C_9H_{12}O_4$ / 188.07
12.28-12.40	Scopolin	$C_{20}H_{18}O_6$ / 355.10
12.78-12.92	Euclernone (Cyclomorusin)	$C_{25}H_{22}O_6$ / 420.19
13.10-13.29	Morusimic acid C	$C_{24}H_{45}NO_9$ / 492.32
13.98-14.07	Morusmic acid F	$C_{18}H_{35}NO_4$ / 330.26
(14.25-14.38)		
14.47-14.94	Mulberrofuran D; 3'-Me ether	$C_{30}H_{36}O_4$ / 462.34
15.68-15.78	Moracin C	$C_{19}H_{18}O_4$ / 334.24*
(15.85-15.96)		
16.08-16.22	Cathafuran C	$C_{24}H_{24}O_4$ / 376.25**
16.31-16.41	Mulberrofuran W	$C_{29}H_{34}O_4$ / 448.30**
16.53-16.66	Alboctalol	$C_{28}H_{24}O_8$ / 490.32
16.80-16.96	2',4',7-Trihydroxy flavanone	$C_{15}H_{12}O_5$ / 275.20
17.07-17.14	3-(3-4-Dihydroxyphenyl)-2 -propeonic acid; Me ester	$C_{10}H_{10}O_4$ / 197.12
17.22-17.36	2-(3,5-Dihydroxyphenyl)-6-hydroxybenzofuran; 3'-O-β-D-Glucoppyranoside	$C_{20}H_{20}O_9$ /404 28

* Na adducts ; ** Not reported earlier from *M. alba*

Table 5: Chemical composition of methanol extract of leaves of *M. alba*

Retention time (m)	Compound	Molecular formula / ion mass
17.43-17.59	Mulberrofuron G (Albanol A)	$C_{34}H_{26}O_8$ / 564.45
18.38-18.46	Benzyl β-D-glucopyranoside	$C_{13}H_{18}O_6$ / 293.21*
19.47-19.54	Pentahydroxy flavone; 3-Me ether	$C_{16}H_{12}O7$ / 316.28
19.62-19.97	Morusiginin A	$C_{18}H_{16}O_6$ / 351.21*
(9.84-19.87		
20.42-20.55) 22.55-22.69	Kuwanon G (Albanin F)	$C_{40}H_{36}O_{11}$ / 694.40
23.05-23.18	Moracin B	$C_{16}H_{14}O_5$ / 289.18
23.47- 23.64	Quercetin-3-O-L-Rhamnoside	$C_{21}H_{20}O_{11}$ / 353.27
23.77-23.89	Moracin T	$C_{15}H_{16}O_9$ / 341.14**
24.19-24.39	Kuwanon L	$C_{35}H_{30}O_{11}$ / 627.28
24.85-25.03	1-(2,4-dihydrophenyl)-2-(3,5,dihydrophenyl) ethelene 3',4-Di-O-β-D glucopyranoside	$C_{26}H_{32}O_{14}$ / 569.28
25.63-25.81	Mulberrofuran F	$C_{38}H_{34}O_8$ / 620.36**
26.83-26.94	Rubixanthin	$C_{40}H_{56}O$ / 553.42
27.75-27.89	Kuwanon A	$C_{34}H_{28}O_8$ / 566.43
27.91-28.11	2-(3-5-Dihydroxyphenyl)-hydroxybenzofuran-3',6-Di-O-β-D-glucopyranoside	$C_{26}H_{30}O_4$ /566.43

* Na adducts; ** Not reported earlier from *M. alba*

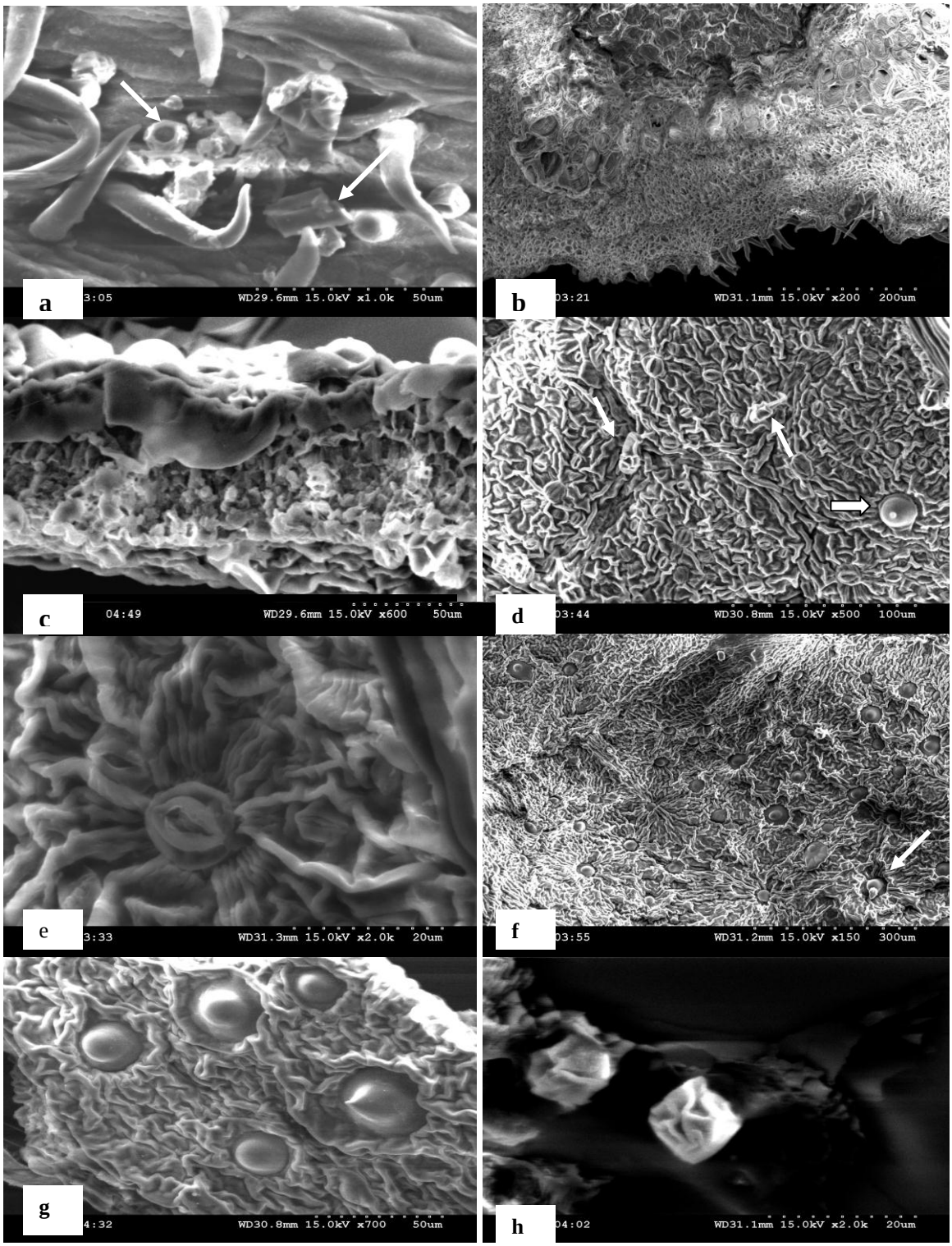

Fig. 1: Scanning Electron Micrographs of various parts of *Morus alba.*
(a) Stem surface (b) Cross section of stem (c) Leaf Cross section (d) Leaf lower surface (e) stomata on leaf lower surface (f) Leaf upper surface (g) Calyx (h) Pollen

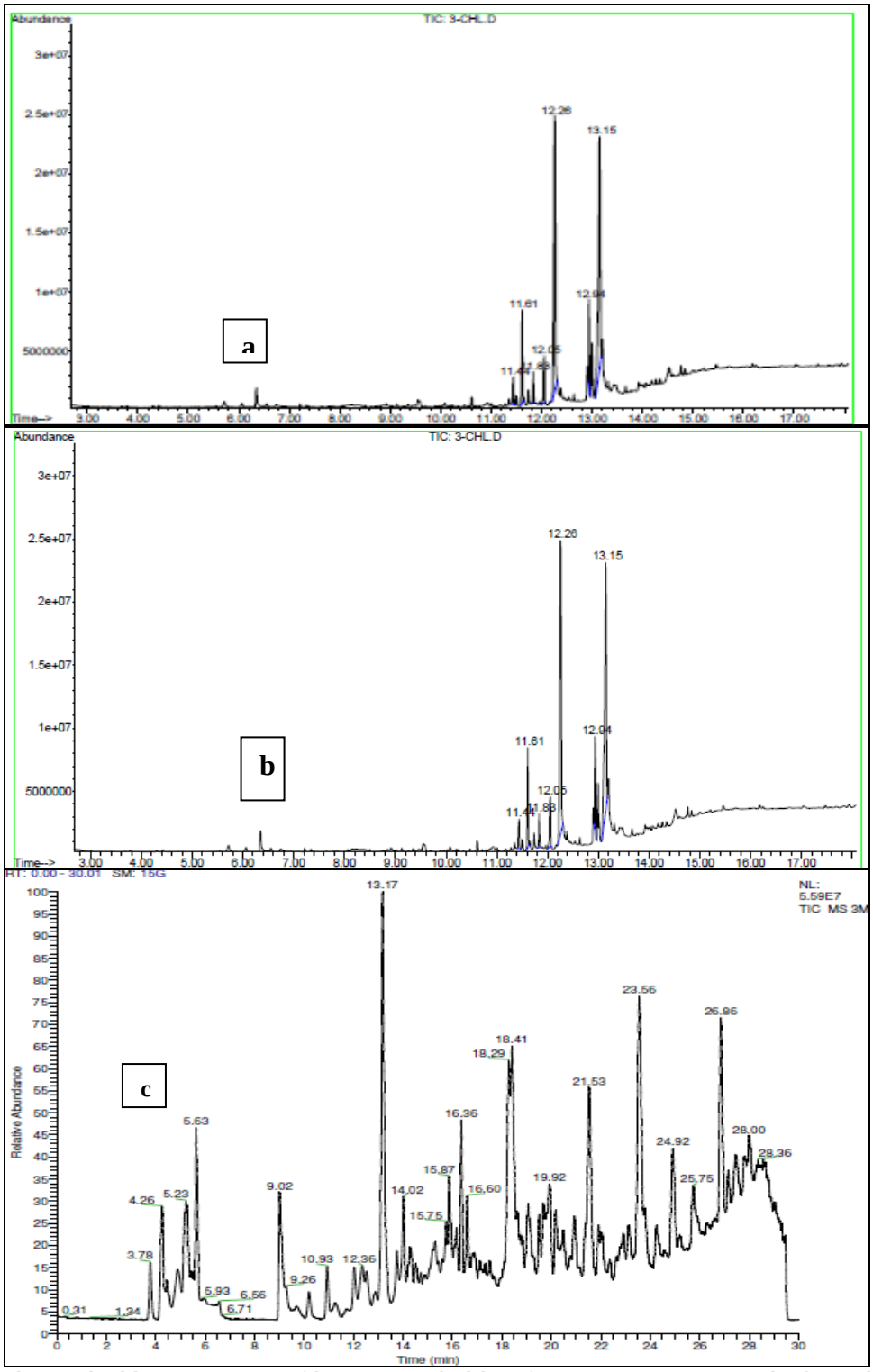

Fig. 2: (a, b) GC spectra of hexane and chloroform extracts respectively and (c) UPLC spectrum of methanol extract of *Morus alba* leaf

REFERENCES

1. Zhishen J, Mengeheng T and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effect in super oxide radicals. Food Chem. 1999; 64:555-559.
2. Kim JW, Kim SU, Lee HS, Kim I, Ahn MY and Ryu KS. Determination of 1-deoxynojirimycin in Morus alba L. leaves by derivatization with 9-fluorenymrthyl chloroformate followed by reversed-phase high-performance

liquid chromatography. J Chromato A. 2003;1002: 93-99.
3. Matsuoka T, Kimura T and Muraoka N. Research of the available constituents from mulberry tree. Tokaku Agri Res. 1994;47:361-362.
4. Onogi A, Osawa K, Yasuda H, Sakai A, Morita H and Itokava H. Flavonol glycosides from the leaves of Morus alba L Shoyagaku Zasshi. 1993;47:423-425.
5. Ercisli S and Orhan E. Chemical composition of white (Morus alba), red (Morus rubra) and black (Morus nigra) mulberry fruits. Food Chem. 2007;103:1380-1384.
6. Doi K, Kojima T, Makino M, Kimura Y and Fujimoto Y. Studies on the constituents of the leaves of Morus alba L. Chem. Pharm Bull. 2001;49(2):151-153.
7. Zeni ALB and Molin MD. Hypotriglycerdemic effect of Morus alba L., Moraceae, leaves in hypeerlipidemic rats. Bra. J Pharmacog. 2010;20(1):130-133.
8. Narendra V and Ameeta A. Pharmacognostical and phytochemical screening of Lantana camara roots. J Phar Res. 2011;4(12):4744-4746.
9. Kumar D, Gupta J, Sunil Kumar, Renu A, Tarun Kumar and Guptha J. Pharmacognostic evaluation of Cayratia trifolia(Linn) leaf. Asian Pacific J Trop Biomed. 2012;6-10 doi: 10.1016/S2221-1691(11)60180-9.
10. Kumar S, Kumar V and Om Prakash. Microscopic evaluation and physicochemical analysis of Dillenia indica leaf. Asian Pacific J Trop Biomed. 2011;337-340. doi: 10.1016/S2221-1691(11)60076-2.
11. Serrano R, da Silva G and Silva O. Application of light and scanning electron microscopy In the identification of herbal medicines. In: Mendéz-villas A, Diaz J (Eds.). Microscopy: Science, Technology, Application and education. Formatex Microscopy Series No 4; 2010;1:182-190. ISBN-13: 978-84-614-6191-2. Spain;
12. Yashvanth S, Rani SS, Rao AS and Madhavendra SS. Anatomical exploration of Leucas aspera (Willd) link a medicinal herb and its pharmacognostic relevance. J Pharm Res. 2011; 4(12):4777-4779.
13. Yashvanth S, Rani SS, Rao AS and Madhavendra SS. Microscopic and micro chemical evaluation (elemental Analysis) of the medicinal herb, Lippia nodiflora (Linn.) Rich (Phyla nodiflora Linn. Green). As Pac J Trop Dis. 2012;2(Suppl 1):S124-S129.
14. Yashvanth S, Rani SS and Madhavendra SS. Microchemical (Elemental) Analysis of Leucas aspera (Wild) Link employing SEM-EDAX. Intl J Pharma Sci Drug Res. 2013;5(1):32-35.
15. Under Wood EJ. Trace metals in human and animal health. J Hum Nutr. 1981;53:37-48.
16. Soderberg T and Halimans G. Treatment of leprosy with adhesive zinc tapes. Lepr Rev.1982;56:271-273.
17. Singh AK, Singh RP and Singh NP. Analysis of Macro and micro nutrients in some Indian medicinal herbs grown in Jaunpur (UP) soil. Nat Sci. 2011;3:551-555.
18. Januja KM. Trace metal elements in Allium cepa (onion) and their therapeutic importance. Hamdard Medicus. 1990;33(2):87-90.
19. Sailey A, Sahu R, Gupta B and Sandhi SM. Analysis of mineral elements of Medicinal plants used for the treatment of asthma, syphilis, diarrhea, skin diseases and rheumatism. Hamdard Medicus. 1994;37:18-22.
20. Sahito SR, Memon MA, Kazi, TG, Kazi GH, Jakhrani MA,Haque QU and Shar GQ. Evaluation of mineral contents in medicinal plant Azadirechta indica (Neem). J ChemSoc Pak. 2003;25(2):139-143.
21. Pirzada AJ, Iqbal P, Shaik W, Kazi TG and Ghani KU. Studies on the elemental Composition and antifungal activity of medicinal plant Lippia nodiflora L. Against skin fungi. J Pak Assoc Dermat. 2005;15:113-118.
22. Rechardt L, Lehtinen S and Wartiovaara J. Scanning electron microscopy and x-ray Microprobe analysis in detection of acetyl cholinesterase in cultured embryonal cancer cells. Histchem Cytochem. 1984;329(11):1154-1158.
23. Rai LC, Jensen TE and Rachlin JW. A morphometric and x-ray energy dispersive approach to monitoring pH altered cadmium toxicity in Anabaena flos-aquae. ArchEnviron Cont Tox .1990; 19:479-487.

24. Pradhan AN, Agrahari AK, Meher A and Mishra MN. Elemental analysis by energy Dispersive x-ray spectroscopy (EDX) of Capparis zeylanica Linn Plant. J Pharma Res. 2010;3(4):669-670.
25. Abdelwahab SI, Abdul AB, Elhassan MM, Mohan S, IbrahimMY, Mariod AA, Alhaz NA and Abdullah R. GC/MS determination of bioactive components and antibacterial properties of Goniothalamus umbrosus extracts. Afr J Biotechnol. 2009;8(14):3336-3340.
26. Gerige SJ, Gerige MKY, Rao M and Ramanjaneyulu. GC-MS analysis of Nigella sativa and antimicrobial activity of volatile oil. Braz Arch Biol Technol. 2009;52(5):1189-92.
27. Derwich E, Chabir R, Taoil R and Senhaji O. In vitro activity and GC-MS studies on the leaves of Mentha piperata (Laminacea) from Morocco. Intl J Pharma Sci Drug Res. 2011;3(2):130-136.39.
28. Kumaresan N, Palanisamy PN, Kumar PE. Chemical investigation of flower of Spathodea companulata by GC-MS. J Nat Prod Pl Resour. 2011;1(2):14-17.
29. Balcke GU, Handrck V, Bergau N, Fichtner M, Henning A, Stellmatch H, Tissier A, Hause B and Frolov A. An UPLC-MS/MS method for highly sensitive high-throughput analysis of phytohormones in plant tissues. Pl Methods. 2012;8:47-57.
30. Engstorm MT, Palijarvi M, Frygnas C, Grabber JH, Muller-Harvey I and Salminen JP.Rapid qualitative and quantitative analysis of proanthocyanidin oligomers and polymers by UPLC-MS/MS. J Agri Food Chem. 2012;62(15):3390-3399.
31. Janakiraman N, Johnson M and Sahaya SS. GC-MS analysis of bioactive constituents of Peristrophe bicalculata (Retz) Nees. (Acanthaceae). Asian Pac J Tropi Biomed. 2012;2S:S46-S49.
32. Patil B and Rajput A. GC-MS analysis of biologically active compounds of chloroform extract of leaves of Butea monosperma. J Pharma Res. 2012;5(2):1228-1230.
33. Sahaya SS, Janakiraman N and Johnson M. Phytochemical analysis of Vitex altissima L using UV-VIS, FTIR and GC-MS. Intl J Pharma Sci Drug Res. 2012;4(1):56-62.
34. Tian CC, Luo YH, Zhao XY, Chen Q, Luo M and Luo LY. Components analysis of volatile matter in semethicone by gas chromatography-mass spectrometry. Afri J Pharm Pharmacol. 2012;6(37):2657-2663.
35. Kenne O, Smyth TJ, Hewage CM and Brunton NP. Antioxidant properties and quantitative UPLC-MS analysis of phenolic compounds from extracts of fenugreek (Trigonella foenum-graecum) seeds and bitter melon (Momordica charantia) fruit. Food Chem. 2013;141(4): 4295-302.
36. Dictionary of Natural Products (DNP) Chapman & Hall/CRC 2010
37. Afendi FM, Okada T, Yamazaki M, Morita AH, Nakamura Y, Nakamura K, Ikeda, S, Takahashi H, Amin MA, Latifah, Darusman, Kazuki Saito and Shigehiko Kanaya. KNApSAcK Family Databases: Integrated Metabolite-Plant Species Databases for Multifaceted Plant Research. Plant Cell Physiol. 2012;53(1):1-12. doi:10.1093/pcp/pcr165.
38. Sandipkumar PB and Pandya SS. SEM and TEM study of bark of Listea chinensis Lam. J Pharma Res. 2012a;5(2):1260-1263
39. Sandipkumar PB and Pandya SS. T.E.M. study of leaf of Litsea chinensis Lam. J Pharm Res. 2012b;5(4):2181-2183.
40. Duke JA. Phytochemical and ethnobotanical databases: Hand book of phytochemical constituents of GRSA herbs and other economic plants. CRC Press. 1992.
41. Patil UH and Gaikwad DK. Effect of varying environmental conditions of mineral status ofstem bark of Anogeissus latifolia. J Pharm Res. 2012;5(2):1140-1143.
42. Yen GC, Wu SC and Duh PD. Extraction and identification of antioxidant components from the leaves of mulberry (Morus alba L.). J Agric Food Chem. 1996;44:1687-1690.
43. Hughes AB, Rudge AJ. Synthesis and biological activity of Deocynojirimycin. Nat Prod Repts. 1994;135.
44. Kumar V and Chauhan S. Mulberry: Life enhancer. J Med Pl Res. 2008;2(10):271-278
45. Park KM, You JS, Lee HY, Baek NI, Hwang JK and Kuwanon G. An antibacterial agent from the root bark of Morus alba against oral pathogens. J Ethnopharmacology. 2003;84:181-185.

46. Geng CA, Ma YB, Zhang XM, Yao SY, Xue DQ, Zhang RP and Chenn JJ. Mulberrofuran G and isomulberrofuran G from Morus alba L.: anti-hepatitis B virus activity and mass spectrometric fragmentation. J Agri Food Chem. 2012;60(33):197-202.
47. Sharma R, Sharma A, Shono T, Takasugi M, Shirata, A, Fujimur T and Machi, H. Mulberry Moracins: Scavengers of UV stress-generated free radicals. Biosci Biotechnol Biochem. 2001;65(6):1402-1405.
48. Yadav AV and Nade VS. Anti-dopaminergic effect of methanolic extract of Morus alba L. leaves. Ind J Pharmacol. 2008;40(5): 221-226.