

both the *kaTapayAdi* and *ArybhaTa*'s systems. This protocol is reliable and free from any kind of defect. It will go a long way in helping researchers in the field of history of Indian mathematics and astronomy, who constantly deal with such decryption processes. The automated process would not only render correctly decoded numbers, but will also speed-up the research work.

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ACKNOWLEDGEMENTS. We thank SASTRA University, Thanjavur for infrastructure, particularly for the purchase of LabVIEW software used in this work.

Received 22 October 2015; revised accepted 16 August 2016

doi: 10.18520/cs/v112/i03/588-591

Mahi: a unique traditional herbal ink of early Assam

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Mahi, a unique herbal ink prepared with cow urine as extractant, was used for manuscript writing in early Assam. The ink had a deep and fast colour and was persistent on Sancipat manuscripts due to its resistance to aerial oxidation and fungi. It was also non-corrosive unlike the corrosive acidic iron gall ink of contemporary Europe. The present study was aimed at analysing the physico-chemical properties of Mahi, including its special properties. The study includes phytochemical analysis, antimicrobial assay, UV–

visible with fluorescence analysis, iron and copper estimation and identification of some polyphenols by HPLC-UV.

Keywords: Ancient manuscripts, physico-chemical properties, spectroscopic analysis, traditional herbal ink.

STUDY of ancient ink and paint may help retrieve useful information regarding traditional practices in addition to unfolding historical mysteries^{1–3}. Modern ink is a complex mixture of pigments, dyes, solvents, resins, lubricants, solubilizers, surfactants, particulate matter, fluorescent and other materials⁴. The primitive Egyptian ink and Chinese ink were carbon-based with the carbonaceous materials obtained from wood tar, burnt bone, lamp shoots, pitch or charcoal. A carbon-based ink, Mashī, was popular in ancient India, except in the eastern part, especially Assam, where an herbal counterpart, called Mahi, was popular till early 20th century AD⁵. An herbal ink, known as gallotannate and iron gall ink (IGI), was widely used in Europe since ancient times till the late 20th century AD⁶. The major ingredients of IGI were tree galls, green vitriol or copperas, gum arabic and water; wine, beer, vinegar and boric acid were also used⁶. A very low pH and excess iron of IGI degraded manuscripts written on paper through acid hydrolysis of the glycosidic bond and through hydroxyl radicals^{4,6}. On the other hand, the nondestructive nature of Mahi is proven by tens of thousands of centuries old Sancipat⁷ (a cellulosic folios made of bark of sancī tree, *Aquilaria agallocha*) manuscripts, a testimony of the rich literary and socio-cultural heritage, still existing in Assam without losing the glaze of the ink (Figure 1 and Figure S1 ([see Supplementary Information online](#)))⁸. For preparation of Mahi, fruit-pulp of hilikha (*Terminalia chebula*), amlakhi (*Emblīca officinalis*) and bhomora (*Terminalia bellerica*), the bark of hilikha, bhomora, mango (*Magnifera indica*), jamuk (*Eugenia jambolana*), bahat (monkey jack, *Artocarpus lakoocha*); and the whole herb of keharaj (*Eclipta alba*), Bar manimuni (*Centella asiatica*) and sharu manimuni (*Hydrocoryl rotundifolia*) were mashed together and soaked in cow urine in a new earthen pot during the foggy winter season and kept away from direct sunlight^{7,9,10}. The raw materials varied depending upon availability¹⁰. Red hot iron tool was dipped into the mixture. Rust of iron nail or blood of kuchiya (*Monopterus cuchia*, a kind of eel) or hirakoch (*Pangasius sutchi*, a kind of cat fish) was also added. Drops of clear Mahi percolate through the bottom of the earthen pot in 9–10 days. There is hardly any scientific report available in the literature on the preparation and properties of Mahi and its possible contribution to the survival of Sancipat manuscripts for centuries in the hot and humid climate of Assam¹⁰. It was therefore thought worthwhile to carry out a scientific study of the composition and physico-chemical behaviour of Mahi to explore the associated traditional knowledge.

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Figure 1. The top page of folio No. 172 from *Adi Dashama* copied with Mahi on Sancipat in AD 1799 preserved at Bengena Ati Satra in Majuli Island of the Brahmaputra. For details see Figure S1 ([Supplementary Information online](#)).

The study was carried out using a sample of Mahi (M1) obtained from a practitioner¹¹ of the manuscript-writing tradition on Sancipat and a fresh model sample of Mahi (M2) prepared under supervision of the practitioner. pH of the Mahi samples after ten times dilution with distilled water was found to be 7.1. The neutral pH of Mahi can be attributed to the absence of any acidic ingredient in its preparation unlike IGI. Due to the neutral pH, Mahi does not corrode the cellulosic Sancipat unlike the corrosion of cellulosic paper by acidic IGI. The phenolic acids of Mahi are also likely to be neutralized by ammonia formed from urea of cow urine¹². M1 showed a partial inhibition against *F. oxysporum* (MTCC-0284) after incubation at 24°C for 48 h and 72 h, where 1 mg/ml of the liquid Mahi sample was used, but did not show any inhibition against *C. albicans* (MTCC-0227) ([see Supplementary Information, Figure S2 online](#)). The antifungal activity of Mahi is expected from those of its raw materials, including cow urine and may have a protective effect on cellulosic Sancipat against fungi abundant in hot and humid climate of Assam¹³.

The sample M2 was prepared following the traditional recipe^{7,9,10} with pulp of hilikha and amlakhi; the whole herb of keharaj and bark of mango tree in equal proportions by weight as herbal ingredients during foggy winter at Tezpur. The ingredients were mashed together and put in a new earthen pot. Then cow urine was added to completely cover the herbal ingredients and a few rusted iron nails were also added to the mixture. The colour of the mixture turned intense blue–black after addition of the nails, which may be attributed to the formation of iron–polyphenol complex. Drops of Mahi percolating down from the pot were collected and preserved in plastic bottles. A part of M2 was centrifuged and dried to powder form in a lyophilizer.

Reaction of Fe(II) with molecular oxygen is considered to cause oxidative decomposition of cellulose by means of highly reactive hydroxyl and hydroperoxide free radi-

icals⁴. Hence, the iron content of M1 was determined using atomic absorption spectroscopy as 5.4 mg/g of the solid sample of Mahi, which is much less than 31.9 mg/g reported for IGI¹⁴. A low iron content of Mahi is expected as only a few rusted iron nails were used for its preparation, unlike IGI where up to 15–20% green vitriol ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was used. The small amount of iron in Mahi remains complexed with polyphenols and may be unavailable for reaction with molecular oxygen⁴. It may be mentioned here that Cu ions from vitriol in IGI are reported to have greater destructive effect on manuscripts than those of iron¹⁵. M1 showed the presence of only 0.2 mg/g of Cu against 4.1 mg/g reported for IGI¹⁴. The trace amount of Cu in Mahi, possibly of cow urine or herbal origin, may also remain complexed with polyphenols and hence cannot degrade the manuscript.

The qualitative phytochemical analysis of the aqueous extracts of powdered M2 showed the presence of flavonoids, tannins, fatty acids and resins as major components; saponins, carbohydrates and steroids as minor components and absence of terpenoids¹⁶ ([see Supplementary Information, Figure S3 online](#)). The sample gave a feeling of soapiness with formation of foam when water was added to it and shaken. The observed surface-active nature of Mahi can be attributed to glycosidic biosurfactants, viz. saponins, which act as solubilizer, stabilizer and binder in Mahi like the gum arabic or animal glue counterparts in other contemporary inks^{4,6}. The total phenolic content in the water extract of lyophilized M2 was found to be 179.8 ± 2.5 μg gallic acid equivalents per gram of lyophilized sample (μg GAE/g) from Folin–Ciocalteu method using gallic acid as the standard¹⁷. The total flavonoid content in the water extract of lyophilized M2 was found to be 579.0 ± 16.4 μg epigallocatechin gallate equivalents per gram of lyophilized sample (μg EGE/g) from aluminum chloride colorimetric method¹⁷. An HPLC analysis of M2 showed the presence of gallic acid (GA), epigallocatechin gallate (EG) and quercetin

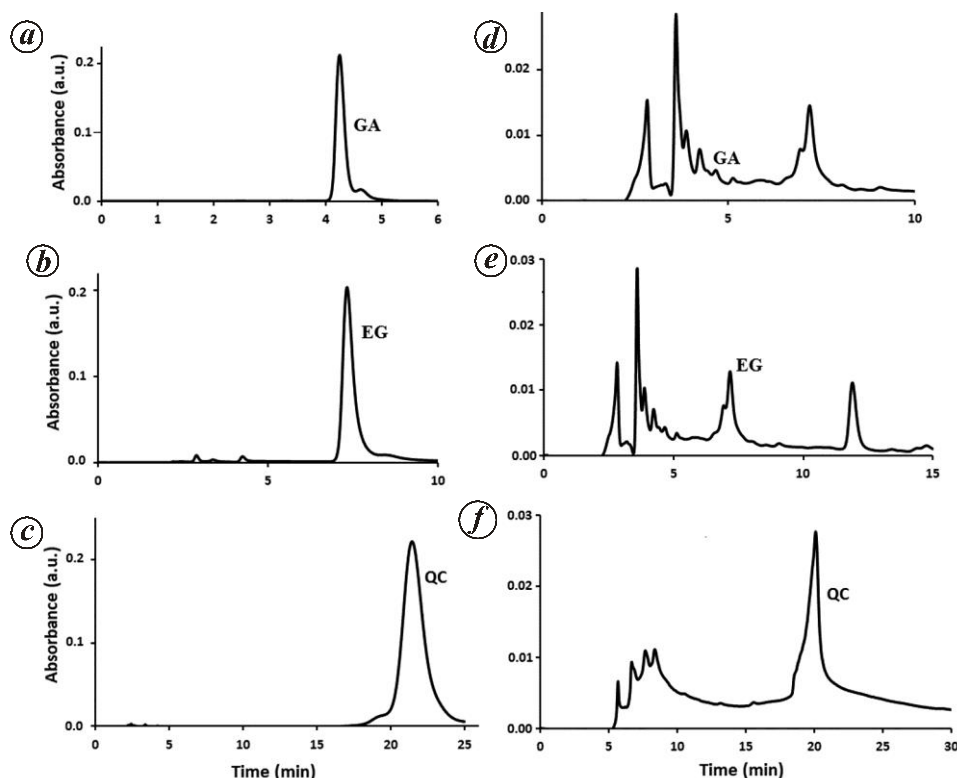


Figure 2. HPLC of (a) gallic acid (GA), (b) epigallocatechin gallate (EG) and (c) quercetin (QC) recorded at 275, 280 and 374 nm respectively; and of M2 recorded for detection of (d) GA, (e) EG and (f) QC at 275, 280 and 374 nm respectively.

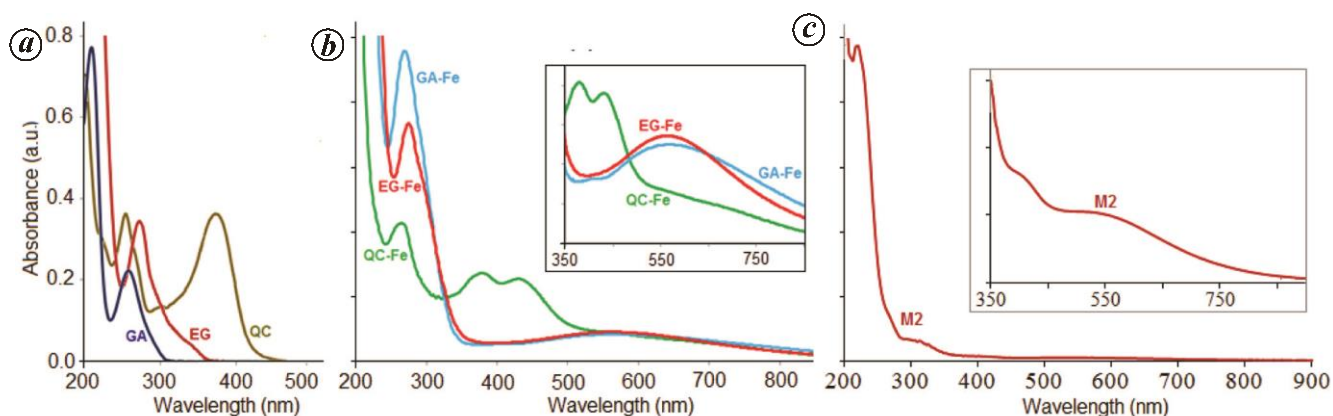


Figure 3. UV-vis spectra of (a) GA, EG and QC, (b) their iron complexes and (c) M2. (Inset) (b, c) Expanded visible spectra of the complexes and M2 respectively.

(QC) (Figure 2). Separation of the compounds was done at the rate of $0.8 \mu\text{l}/\text{min}$, in isocratic elution mode, using 0.1% acetic acid in 69.9:30 water:methanol solvent. Quantitative estimation showed the presence of small amounts of GA compared to EG, with QC showing maximum value.

Figure 3 shows the UV-visible spectra of M2 along with those spectra of its constituent polyphenols and their complexes with iron⁶. A flat absorbance of M2 in the visible region can be observed as expected from the black

colour of the ink. A broad shoulder from ≈ 420 to 750 nm, which gradually decreases with increasing wavelength can be attributed to the iron complexes of the polyphenols, including GA, EG and QC⁶. It is interesting to note the use of rusted iron or blood of some fishes as the source of iron ions to intensify the colour of Mahi. Though the ink is intense black, it turns reddish-brown in very dilute aqueous solution ([see Supplementary Information, Figure S4 online](#)). The appearance of reddish-brown colour in highly diluted Mahi may be attributed to

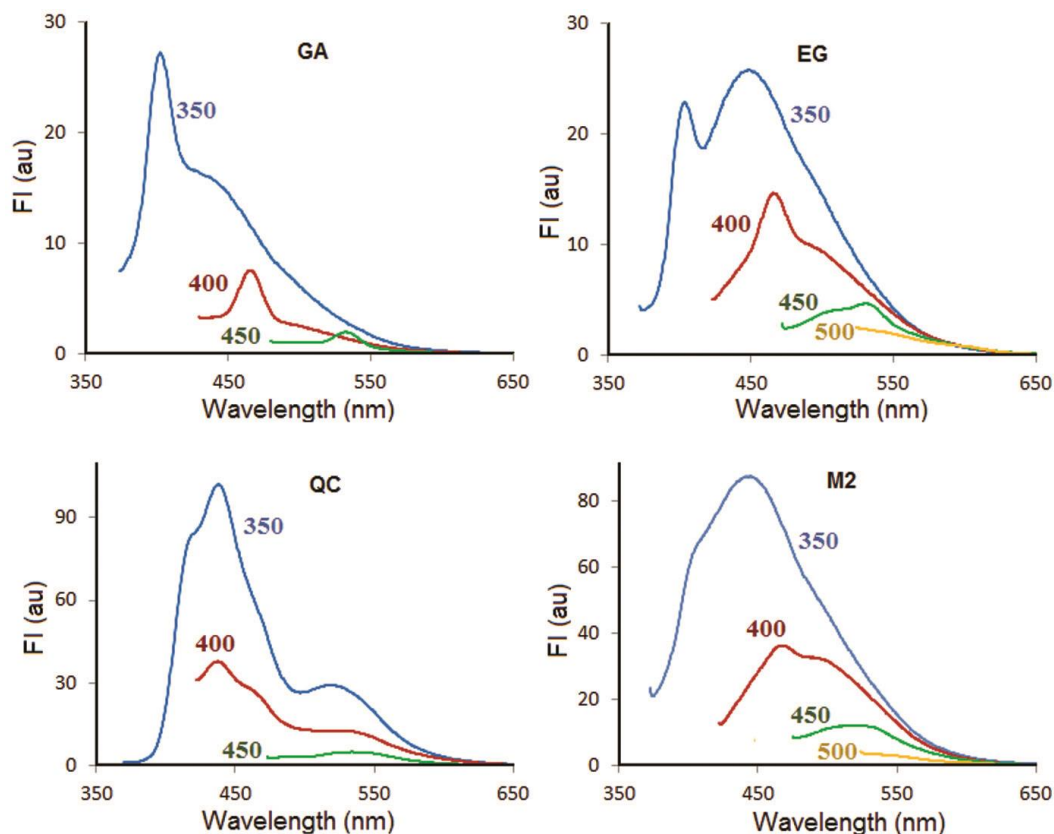


Figure 4. Fluorescence spectra of GA, EG, QC and M2 recorded with excitation at 350, 400, 450 and 500 nm.

the gradually decreasing absorbance of the ink with increasing wavelength.

Mahi shows fluorescence activity on irradiation of UV light as well as visible light up to ≈ 500 nm (Figure 4), as expected from the reported fluorescence activity of its polyphenolic components, e.g. GA, EG and QC. Figure 4 shows the fluorescence spectra of these polyphenols along with those of M2 recorded after excitation at 350, 400, 450 and 500 nm. We did not observe any fluorescence of the ink when excited above 500 nm, which can be attributed to the decrease in fluorescence activity upon complexation¹⁸. The observed reddish-brown colour of very dilute Mahi may also be attributed to fluorescence intensity of Mahi, which increases gradually with decreasing wavelength.

This study on Mahi provides insight regarding its composition, and physico-chemical and biochemical properties. The major phytochemical constituents in Mahi have been identified as phenolic acids, flavonoids and tannins. It has been observed that a small amount of iron sourced from rusted iron nails intensifies the colour of the ink by forming iron–polyphenol complex, thus imparting intense black colour to Mahi. The Sancipat manuscripts written with Mahi are free from destructive effects of acid hydrolysis, oxidative decomposition by Fe or Cu, and fungus, enabling them to survive for centuries in harsh

climate, unlike paper manuscripts written with IGI. Fluorescence of the uncomplexed polyphenols also contributes to the glaze. *Glycosidic biosurfactants* of herbal origin act as solubilizer, stabilizer and binder in Mahi, like gum arabic or animal glue counterparts in other contemporary inks. An observed, partial antifungal activity of Mahi may have a protective effect on Sancipat manuscripts. Thus, the present study has shown some interesting facts about the composition, lustre, longevity, antimicrobial activity and non-corrosiveness of Mahi. Further work is in progress on upscaling the preparation of Mahi, quantitative estimation of its chromogenic and bioactive compounds, and detailed spectroscopic analysis.

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ACKNOWLEDGEMENTS. R.K.D. thanks M. M. Bora, R. D. Goswami (Tezpur University) and D. Nath (Dibrugarh University) for help and useful discussions. B.R.G. thanks Tezpur University for fellowship.

Received 19 April 2016; revised accepted 30 August 2016

doi: 10.18520/cs/v112/i03/591-595

Climate change-driven shifts in elevation and ecophysiological traits of Himalayan plants during the past century

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As broad-scale distributions of plants are shaped by climatic conditions, changes of climate necessarily result in shifts of distributional limits. These shifts are closely coupled with changes in plant ecophysiology, growth and productivity. Among environments subjected to the highest increase in temperature in the last decade and the greatest expected warming predicted for the future, high-mountain biomes belong to the most frequently considered. Evidence for distributional shifts has been mostly documented in European and American mountains, while the largest and highest mountainous areas are located in Asia. The present study aims to detect climate change-driven shifts in elevation and ecophysiological traits of endemic herb species of Himalaya with the help of herbarium specimens as potential tool. We observed significant rapid upward elevational shift of 55.2 m/decade compared to average global shifting of 6.1 m/decade and impulsive variations in secondary metabolite concentration. Significant negative relationship was found for stomatal density, $\delta^{13}\text{C}$ with the lapse of years. Analysis of instrumental temperature data reveals an increase of 0.31°C in mean maximum and 0.79°C in mean minimum temperature during the last century.

Keywords: Climate change, distributional shift, $\delta^{13}\text{C}$, Himalayan plants, metabolites, stomatal density.

LONG-TERM observations, experiments and modelling studies have demonstrated significant changes in patterns of global biodiversity in response to climate change^{1–4}. In particular, mountainous regions are predicted to be more vulnerable for biodiversity loss due to fragmented ecosystems⁵. Recently, upward shifting of vegetation zones,

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