Table 2. Flavonoidal content in the leaf extract of *Fagonia cretica*

	Free quercetin	Bound kaempferol	Total content
	1.89	0.88	2.77
	1.92	0.91	2.83
	1.88	0.94	2.84
Average	1.89	0.91	2.81
S.D.±	0.02	0.03	0.03

more effective against the organism. The highest inhibition of the organism was observed with *F. cretica* leaf extract, which was more effective than antibiotic tetracyclin (Table 1).

Considering the remarkable results with *F. cretica* leaf extract, phyto-chemical analysis was carried out in leaf extract (Table 2). For phyto-chemical studies, leaves were extracted with hot ethanol (80% 100 ml/g dry wt), filtered and the filtrates concentrated *in vacuo* separately. Each of the residues was re-extracted with petroleum ether, ethyl ether and ethyl acetate (fractions A, B and C) in succession[6]. Various ethyl acetate fractions (C) were hydrolysed (in 7% H_2SO_4, 2 h) and neutralized. Fraction A was rejected as being rich in fatty substances, whereas fraction B was analysed for free flavonoids and fration C for bound flavonoids. The ethyl ether and etheyl acetate (hydrolysed) fractions were separately pooled for further analysis. Each of the isolates was examined by thin layer chromatography (TLC, silica gel-g coated plates) along with the standard reference samples of apigenin, isorahm netin, isovitakin, kaempferol, lucteolin, myrecitin, quercetin and vitexin. The flavonoids were separated by TLC using *n*-butanol–acetic acid–water (4 : 1 : 5 upperlayer) as solvent system, observed under UV light, placed in a chamber saturated with ammonia and were sprayed with 5% ethanolic ferric chloride.

The compounds were removed from unsprayed TLC plates (observed under UV light) along with the silica gel. The mixtures were separately extracted with ethanol and the compound dried. The isolated compounds were crystallized separately in 50% ethanol and subjected to IR and UV spectra studies with the known flavonoid to make its presence sure. The presence of flavonoids in leaves of *F. cretica* confirmed its anti-bacterial potential, as flavonoids are said to be antimicrobial in nature[7].

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Tissue culture of *Bulbothrix setschwanensis* (lichenized ascomycetes) *in vitro*

The tissue culture of several lichen taxa has been successfully achieved[1]. An attempt has been made here to derive cultures from the natural thalli of a lichen species *Bulbothrix setschwanensis* (Zahlbr.) Hale.

The lichen *B. setschwanensis* (Zahlbr.) Hale, producing atranorin and salazinic acid under natural conditions was used for this study. A part of the material has been preserved as herbarium specimen (India, Uttar Pradesh; Kumaon Hills, Nainital, Hanumangarh, alt. ca. 1900 m. about 3.22 km from Nainital, on trees, *U.V. Makhija*, 97.130-**AMH**).

Lichen culture was started a few days after collection of the lichen. The methods used for the culture are similar to those as reported by Yamamoto, Mitzuguchi and Yamada[2].

The culture media were selected to be nutrient-poor so as to ensure a proportionate growth of both the photobiont and the mycobiont to enable the formation of cell aggregates.

The following culture media were used: 1) malt–yeast extract medium (MY) containing malt extract 10 g, yeast extract 4 g, agar 15 g in 1 l of water; 2) water–agar medium, agar 15 g in 1 l of water; 3) Malt Extract Broth (MEB), 20 g per l of water; 4) Bold's Basal Medium (BBM)[3] and 5) Modified Bold's Basal medium. The composition of modified (by us) BBM is given in Table 1.

All chemicals used in this experiment were of analytical reagent grade (Hi-media Laboratories Pvt Ltd, India). The pH of all media used here was adjusted to 6.6 with 1 N NaOH or 1 N HCl .

About two months after incubation, the most actively growing tissues composed of mycobiont without contamination were selected and cut into smaller masses of tissue with the scalpel. Each of these masses was transplanted onto fresh media and was cultured under the same conditions as mentioned earlier. The trans-

plantations were repeated after every three months.

The development of photobiont and mycobiont was observed with binocular-stereo and transmission light microscopes and recorded by macro and microphotography. Lactophenol was used as the mounting medium.

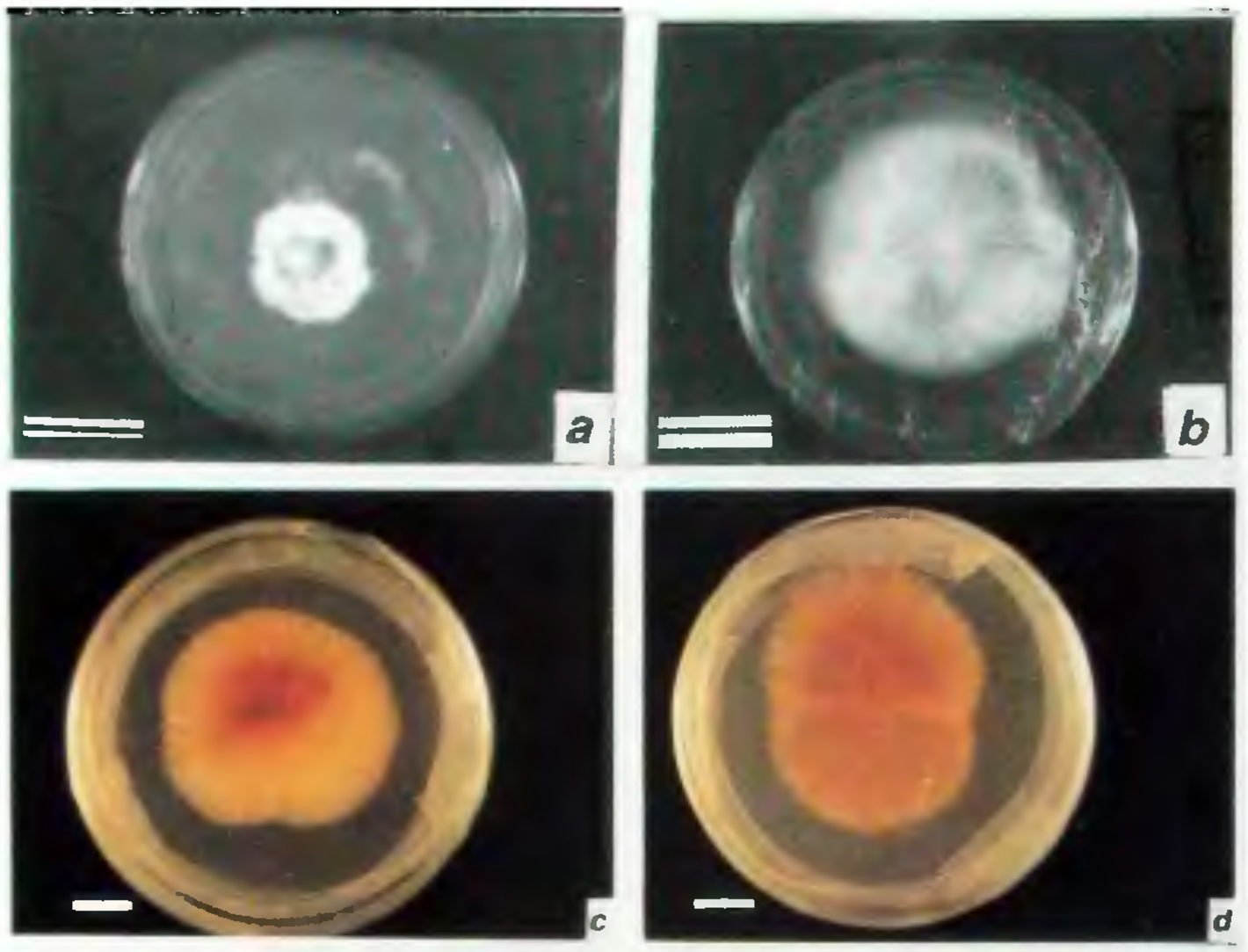

Figure 1. Tissue obtained from the lichen thallus of *B. setschwanensis in vitro*. *a* and *b*, Radially developed fungal mycelium on modified BBM; *c*, Yellow colouration to the culture; *d*, Culture turning green; *e* and *f*, Formation of cleavage in the culture composed of both mycobiont and photobiont; *g* and *h*, Cell aggregates composed of colourless fungal hyphae and dark green algal cells in groups (micropreparations from the same culture). Scale: *a–f*, bar = 2 cm; *g* and *h*, bar = 20 µm.

The chemical data have been obtained by the standardized method of TLC[4] by using standard solvent systems BDA (benzene : dioxane : acetic acid, 180 : 45 : 5, 230 ml) and HEF (hexane : ethylether : formic acid, 130 : 80 : 20, 230 ml). The identification of lichen substances was made by comparison with the standard lichen substances and samples of several species containing atranorin, norstictic and salazinic acids and corresponding natural thallus.

The initial growth of mycobiont hyphae was obtained radially from the inoculated lichen segments after one week in BBM and two weeks in MY. However, the percentage of contamination was less and at lower rate in petri plates (75%) and in conical flasks (62%). When compared to MY the growth was much faster in BBM.

The cell aggregates which began to form about three to four days after transplantations on BBM and 12 days after transplantations on MY were again composed of fresh hyphae only. Out of the many culture media tested, the cell aggregates composed of both the symbionts having fresh hyphae and the algal cells (Figure 1 *g* and *h*) were obtained only on modified BBM about 8–12 weeks after transplantation from BBM. The cell aggregates thus obtained on modified BBM were initially snow-white in colour (Figure 1 *a*). They started growing radially from the subcultured segments of initial tissues and covered three-fourth of the area of the petri plates (Figure 1 *b*) and conical flasks. Bright yellow colour appeared (Figure 1 *c*), which eventually turned into green (Figure 1 *d*). At this stage algal cells were found to be surrounded by fungal hyphae and after 3–4 months they were found to produce lichen substances salazinic acid and atranorin as in the natural thallus.

After six months, the circular growth was divided into two more or less equal halves by the formation of a cleavage from the periphery to the centre (Figure 1 *e* and *f*).

Thus the tissue of *B. setschwanensis*, composed of mycobiont and photobiont producing atranorin and salazinic acid can be cultured on modified BBM after the induction of initial growth of fresh hyphae from the fragments of natural thallus on BBM[3]. Cultures can be maintained at 18°C with an 8 h period of light (400 lux) alternated with a period of 16 h dark and 50–80% relative humidity in culture room.

Table 1. Composition of modified BBM

Part I	Gram per litre solution
$NaNO_3$	3.0
$MgSO_4.7H_2O$	1.5
KH_2PO_4	2.0
$CaCl_2$	0.1
K_2HPO_4	3.0
NaCl	0.1
	(10 ml of each stock solution to 940 ml of distilled water)
Part II	**Gram per litre solution**
1. H_3BO_3	11.42
2. $FeSO_4.7H_2O$	0.82
$ZnSO_4.7H_2O$	0.40
$MnCl_2.4H_2O$	0.43
3. $(NH_4)_6MoO_{24}.4H_2O$	0.20
$CuSO_4.5H_2O$	1.57
$Co(NO_3)_3.6H_2O$	0.98
4. EDTA	15.00
KOH	3.10
	(1 ml of each stock solution to the part I solution)
Agar	15.0
Glucose (D-glucose)	10.0

Salazinic acid was not detected in mycobiont or photobiont cultures of *B. setschwanensis*. It was detected only in cultured tissues which were composed of both bionts. We believe that the production of salazinic acid in cultured *B. setschwanensis* tissues is based on a relationship between mycobiont and photobiont and plays an important role in the production of salazinic acid.

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Earliest complex life on earth: New look from fossil discoveries

One of the instructive examples of controversy regarding the time of the earliest evolution of complex life on earth may be seen with the one billion-years or more (Proterozoic) age for the multicellular worm-like trace fossil[1] and the Cambrian, supposedly 545 m.y. old, tiny-shelled animal fossil[2] findings from the lower Vindhyan rocks in central India. The sharp contrast in ages inferred seems to collectively call for reassessment of the age of the Vindhyan rocks itself, and eventually a new look into the explosion of early life having complex form. But now it may not be a serious problem in the light of the following. Firstly, in the seventies and eighties too, the impressions of animal fossils belonging to the annelids were discovered from the Rohtas Formation, lower Vindhyan[3] and from the much older Bijawar Group rock (around 2000 m.y. old)[4]. Particularly, the metazoan trace fossils in Bijawar were examined by A. Seilacher in the eighties. Hence, the much talked about report of multi-cellular Proterozoic life[1] in the late nineties is not new enough to be considered astounding. Secondly, the evolution of complex life during Proterozoic is further corroborated by another noteworthy record of spongy body-fossil, possibly of animal origin, from the Semri Porcellanite Formation, Kheinjua Shale Formation and Rohtas Limestone Formation of Vindhyan[5]. These remains show psilate or perforated body of triangular to subtriangular shape. Although these discoveries cannot be underrated, there still persists some speculation on the less likely probability of 'backing of life's clock by a large span of time'. At present, this is uncalled for, because it is logically deduced that these recent discoveries clearly confront with the conventional viewpoints on the Cambrian life explosion[6], which invariably also helps to a great extent to rule out suspicion on the Proterozoic age of the lower Vindhyan, primarily by means of some modern geochronological data. Thus, a major fret about the want of modern radiometric dating expressed in various critiques[7–9] and subsequent discussions[10,11] may not be of crucial significance while appraising the earliest complex life. Yet, in this respect some of the opinions, which have been seriously placed in these critiques on the recent fossil discoveries, deserve scrutiny; so that the opinions rendered are not misconceived during a healthy debate on the earliest evolution of the complex life and also future research necessitating age revision of the Vindhyan, mainly on the basis of tiny-shelled animal fossils.

According to Brasier[7] there could be a big gap in age between the Kheinjua Sandstone (1.1 b.y.), containing fossilized worm burrows, and the overlying