Table 2. Vegetative voltaic cells using different plants*

Plant material	pH of decoction	Potential difference (V)	Conductivity (mmhos)	Current (mA)
Bryophyllum calycinum	4.63	3.4–3.2	2.8	302.0–300.0
Basella rubra	5.19	3.1–3.0	4.5	297.5–280.0
Barleria cristata	6.82	3.0–2.9	4.6	295.0–285.0
Adhatoda vasica	7.52	2.8–3.1	4.2	267.0–260.0
Mixture of above (1 : 1 : 1 : 1)	5.43	3.0–3.3	3.8	286.0–278.0

*Plant decoction contains 1% borax to prevent microbial contamination.

especially from plant leaves. Plant leaves are abundantly available in the vegetative world.
(ii) The mechanism has the simplicity of a voltaic cell. It has no harmful effects, is free from environmental pollution hazards and can be operated by anyone.
(iii) The device can generate power from leaves of any plant, including weeds. Any part of a plant such as the soft stem, root, fruit juice, leaf and algal material can be used for this purpose.
(iv) The vegetative voltaic cell can be re-used by changing the leaf decoction. Again, by replacing any part of the device, it may further be repeatedly used. Here, the corrosive effect of the energy source is nominal, so the electrodes remain in good condition for a long duration. As the electrolytes are mostly weak organic ions, corrosion would be much less than in the case of strong electrolytes. One of the electrodes is carbon, which is not affected by corrosive action. The other one is zinc, which however is susceptible to a certain extent.
(v) The plant decoction was treated with Na-benzoate or borax to prevent microbial contamination, as mentioned in the text. At room temperature, no change is observed for 5–6 days. If stored in a refrigerator along with the preservative, no marked change is observed for eight months.
(vi) In a voltaic cell, polarization effect is one of the problems to produce power, because a very thin layer of hydrogen gas is wrapped over the cathode. As a result, the potential difference becomes gradually reduced. In this case the balance of natural oxidants and reductants present in plant leaves presumably nullifies such effects, leading to better results.
(vii) Here, power is generated from plant sources on the principle of voltaic or electrical cell, and there is no impact of sunlight. So power can be obtained during the day or night (in light and darkness) on a regular, continuous basis.

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Ethanolic extract of *Bacopa monniera* (Brahmi) induces shortening of cell-cycle durations in naturally synchronous *Physarum polycephalum*

Plants have been used as sources of medicinal and pharmaceutical agents in the form of isolates, extractives or as lead compounds for synthetic optimization. Vinca alkaloids, Epipodophyllotoxins, Taxanes and Camptothecin represent different classes of plant-derived anticancer agents that continue to be an important component of modern pharmaceuticals[1].

Bacopa monniera L. (Scrophulariaceae), commonly known as Brahmi, is a creeping herb with a bitter taste found throughout India in damp and marshy areas. It has been used in folklore medicine and the ancient traditional system of Ayurveda as a nerve tonic for improvement of intelligence, memory and revitalization of sense organs, to treat epilepsy, insomnia, asthma, rheumatism and also as a diuretic and cardiotonic[2–4]. Several phytochemical studies have been carried out on the plant in Indian laboratories, and it is known to contain nicotine, brahmine, herpestine, hersaponine, bacosides A, B, C, D and other chemicals like stigmastanol, b-sitosterol and stigmasterol[2,5–8]. In pharmacological studies, alcoholic extract of *Bacopa* has been shown to possess anticancer activity against Walker carcinosarcoma 256 in rats[9], growth-inhibitory effects on Sarcoma 180 cultures[10], activity affecting avoidance response in rats[11–13], and a potent antioxidant activity[14].

Here, we report the phase-specific effects of Brahmi on cell-cycle durations in the lower eukaryotic myxomycete fungus, *Physarum polycephalum*. In surface cultures of this syncytial organism, over a million nuclei divide in perfect natural synchrony, making it an ideal model system for such studies.

B. monniera plants were collected from the botanical garden at the University of Calicut during February and March 2002. Authenticated fresh plants were ground in a mortar, defatted with petroleum ether (1 : 10 w/v; 60–80°C) and filtered. The residue was then Soxhlet extracted with 80% ethanol (1 : 10 w/v) for 12 h. The extract was evaporated to dryness (yield:

5.9% w/w) and dissolved in dimethyl sulphoxide (DMSO).

The M3C, VIII strain of *P. polycephalum* (McArdle Cancer Laboratory, University of Wisconsin) was maintained in the dark at 24°C as microplasmodial suspension cultures in a semi-defined medium (SDM)[15]. Surface macroplasmodia were prepared by the coalescence of microplasmodia on Whatman No. 40 filter paper[16]. Rhythmic synchronous mitoses, referred to as post-fusion mitoses (PFM) were observed in the macroplasmodia at regular intervals of 10±1 h. A set of surface plasmodia used in a particular experiment, prepared from pooled microplasmodial cultures to ensure synchrony among them, are called 'sisters'. Phase-contrast microscopy of ethanol-fixed smears prepared from macroplasmodial explants was carried out for the determination of mitotic stages[17]. G1 phase is absent and S phase, which starts during the telophase of mitosis in this organism, lasts for about one-third of the total cell-cycle duration[18] (~ 3.3 h in a 10 h cycle). The syncytial macroplasmodia which attain a size of about 4–5 cm by the II PFM, a 'giant cell', were cut along with the supporting filter paper into a number of sectors. Untreated control sectors were maintained in SDM and the experimental sectors in SDM containing 100, 200 and 750 μg of the drug, respectively in Petri dishes; appropriate controls were kept in SDM containing equivalent amounts of the solvent DMSO. After a 2 h pulse, traces of the drug-containing medium were removed from plasmodial sectors by blotting them on sterile filter papers followed by two 5-min changes (washes) in drug-free SDM. Thereafter the sectors were maintained in SDM until the III PFM was observed in them. Sister plasmodia were used to cover the cell-cycle between the II PFM and III PFM.

The cell-cycle durations were significantly affected by Brahmi extracts depending on both the phase of the cell-cycle at which the drug was administered as well as the concentration of the drug. The results of a representative experiment are given in Figure 1. The time of control metaphase is denoted as '0' point according to convention. A significant advancement of the initiation of synchronous mitosis was observed in sectors which received drug treatment at low concentration (100 μg/ml SDM) during early S and late S by 1 h 35 min and 1 h 15 min respectively, compared to control. This effect was also seen, to a lesser extent, in early G2-treated sectors (~ 20 min). At 250 μg/ml SDM, though the S phase-treated sectors still showed the maximum advancement (1 h over control), a drastic reduction in this effect was observed in late S-treated ones; the entire G2 phase was found to be refractory to the drug. At 750 μg/ml SDM, however, the advancements were still evident, but were relatively far less in magnitude. Interestingly, at this concentration mitosis was delayed in sectors exposed to the drug during early G2 only. At all concentrations tested, the mid and late G2 phases notably were found to be insensitive to the mitosis-advancing/delaying effect of the drug.

A gradual loss of the mitosis-advancing/delaying activity of Brahmi was observed when the stock solution was maintained at 4°C. The results of a set of experiments at a dose of 100 μg/ml are shown in Figure 2. Maximum activity was observed within a week of extract preparation. By about a month, the activity was found to be completely abolished.

The present study reveals the cell-cycle phase-specific effects of Brahmi in a normal eukaryotic system, although the anticancer activity of the extract against Walker carcinosarcoma 256 in rats[9] and growth-inhibiting activity as observed in cultured sarcoma cells[10] has been reported. In a rat liver-based *in vitro* system, the extracts have also been shown to possess a potent antioxidant activity[14]. Our study has been particularly successful in revealing the ability of the ethanolic Brahmi extract to advance the initiation of mitosis at low doses (100 and 250 μg/ml SDM), resulting in a 10% overall reduction in cell-cycle duration. Moreover, when the dose is increased from 100 to 250 μg/ml SDM, the advancing effect is seen to decrease, following almost an apparent inverse relationship with respect to the cell-cycle phases from S towards M.

One of the major components of the ethanolic extract of Brahmi has been shown to be bacoside A[6,7], known to enhance protein kinase activity in rat hippocampus[13]. As this class of enzymes includes the key regulators of signal transduction pathways associated with cell-cycle progression[19], the likelihood of their involvement in shortening the cell-cycle duration cannot be ruled out. Since at a higher dose of 750 μg/ml SDM, the extract induced a mitotic delay, albeit only in the early G2 period, it appears that higher levels of some component(s) of the extract might be inhibiting processes occurring during this part of the cell-cycle. It is pertinent to note here that shorter than normal mitotic cycles have been observed in *Physarum* plasmodia after the first UV-induced delayed mitosis, as a response to balance the disturbed nucleo-cytoplasmic ratio due to the breakdown of nuclei induced by radiation[20]. Over-expression of mitotic cyclins has also been shown to bring about advancements in the timing of mitosis in yeast[21]. Further, studies need to be carried out not only to identify the components of the extract responsible for the

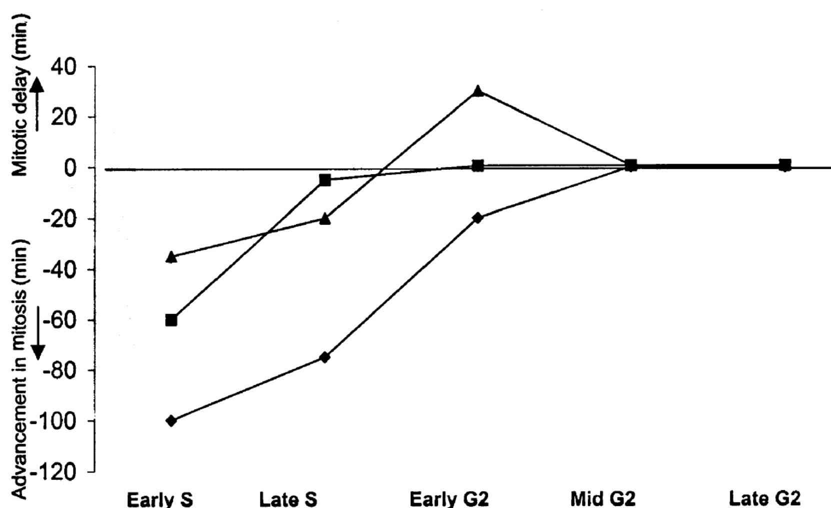

Figure 1. Phase-specific effects of Brahmi extract on cell-cycle of *Physarum polycephalum*. –◆–, 100 μg/ml; –■–, 250 μg/ml; –▲–, 750 μg/ml. Control metaphase is denoted as the '0' point.

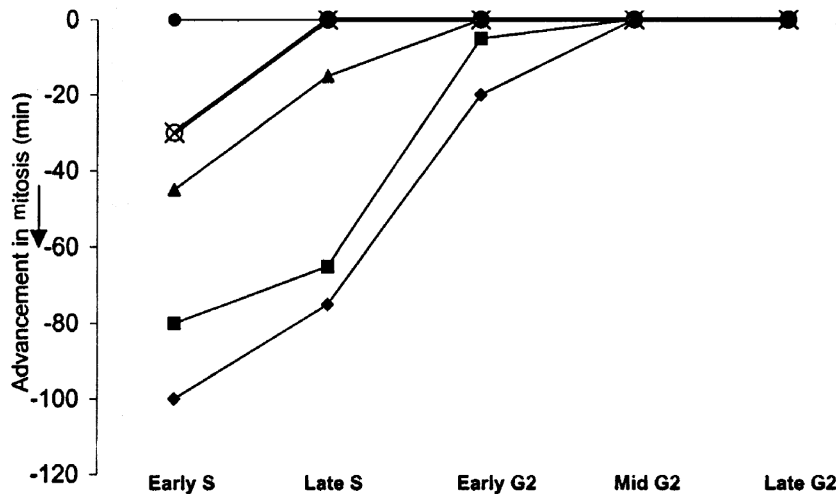

Figure 2. Gradual loss of activity of Brahmi extract on storage. –◆–, 1st day; –■–, 7th day; –▲–, 15th day; –⊖–, 23rd day; , –✳– 30th day; –●–, 35th day. Control metaphase is denoted as the '0' point.

observed effects, but also to study the mechanism of their action on cell cycle stage-specific processes. This should also give us a better insight into the role, if any, played by synergism in the overall action of the component(s) of the extract on the cell.

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Role of plant proteins in formulated fish feeds

Sustainable fish culture is a formulated feed-based industry. Fish feeds constitute the major fraction of the operational cost in both intensive and semi-intensive culture systems globally. Protein is the major item of formulated feeds. It is required in large quantity by many cultivable fishes. Protein requirement of fishes is uniformly high irrespective of their food habits and ranging from 35 to 70% dry weight of the feed. Growth in cultivable fishes is primarily influenced by quality (amino acid composition) and quantity of proteins in the formulated feeds, compared to other farmed animals. Conventional fish meal continues to be a primary protein source in formulated feeds. But its rising cost, uncertain availability and unreliable quality have led to the scientific search for alternative sources. The utility of plant protein sources (PPS) to completely or partly replace the fish-meal is being researched meticulously. However, studies reveal negative results as reduced specific growth rate (SGR)[1–3] and reduced protein efficiency ratio (PER)[1,4–6], when PPS exceed identified levels in formulated feeds. Briefly, the balanced fish feeds to enhance maximum growth in most of the cultivable