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ON ATTRACTION OF SLIME MOULD PHYSARUM POLYCEPHALUM TO PLANTS WITH SEDATIVE PROPERTIES

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Abstract. A plasmodium of acellular slime mould *Physarum polycephalum* is a large single cell with many nuclei. Presented to a configuration of attracting and repelling stimuli a plasmodium optimizes its growth pattern and spans the attractants, while avoiding repellents, with efficient network of protoplasmic tubes. Such behaviour is interpreted as computation and the plasmodium as an amorphous growing biological computer. Till recently laboratory prototypes of slime mould computing devices (Physarum machines) employed rolled oats and oat powder to represent input data. We explore alternative sources of chemo-attractants, which do not require a sophisticated laboratory synthesis. We show that plasmodium of *P. polycephalum* prefers sedative herbal tablets and dried plants to oat flakes and honey. In laboratory experiments we develop a hierarchy of slime-moulds chemo-tactic preferences. We show that Valerian root (*Valeriana officinalis*) is strongest chemo-attractant of *P. polycephalum* outperforming not only most common plants with sedative activities but also some herbal tablets.

Keywords: Physarum polycephalum, slime mould, valerian, passion flower, hops, vervain, gentian, wild lettuce, chemo-attraction

1. Introduction

Physarum polycephalum belongs to the species of order *Physarales*, subclass *Myxogastromycetidae*, class *Myxomycetes*, division *Myxostelida*. It is commonly known as a true, acellular or multi-headed slime mould. Plasmodium is a 'vegetative' phase, single cell with a myriad of diploid nuclei. The plasmodium looks like an amorphous yellowish mass with networks of protoplasmic tubes. The plasmodium behaves and moves as a giant amoeba. It feeds on bacteria, spores and other microbial creatures and microparticles (Stephenson & Stempen, 2000). Being placed on an appropriate substrate, the plasmodium propagates, searches for sources of nutrients and follows gradients of chemoattractants, humidity and illumination. The plasmodium spans sources of nutrients with a network of protoplasmic tubes similar to but not perfectly matching, graphs from the family of β-skeletons (Adamatzky, 2008).

Due to its unique features and relative ease of experimentation, the plasmodium has become a test biological substrate for implementation of various computational tasks. The problems worked out by the plasmodium include maze-solving, calculation of optimal graphs, construction of logical gates, sub-division of spatial configurations of data points, and robot control, see e.g. (Nakagaki et al., 2001; Shirakawa et al., 2009; Tsuda

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et al., 2004; Schumann & Adamatzky, 2011; Adamatzky, 2010a). The plasmodium's behaviour is determined by external stimuli and excitation waves travelling and interacting inside the plasmodium (Nakagaki et al., 1999). The plasmodium can be considered as a reaction-diffusion (Adamatzky, 2007) or an excitable (Achenbach & Weisenseel, 1985) medium encapsulated in an elastic growing membrane.

In (Adamatzky, 2010a) we introduced a concept of Physarum machines. A Physarum machine is a programmable amorphous biological computer experimentally implemented in plasmodium of *P. polycephalum*. A Physarum machine on a nutrient-rich substrate behaves as an auto-wave in an excitable medium. On a non-nutrient substrate it propagates similarly to a wave fragment in a sub-excitable medium. A Physarum machine can be programmed by configurations of repelling (salt) and attracting (food) gradients, and localised reflectors (illuminated obstacles). Gradients fields generated by discrete configurations of attractants are an important prerequisite for successful programming of Physarum machines.

Studies on attractants of *P. polycephalum* can be traced back to early 1900s, with interest usually reignited every 20-30 years, see overview of pre-1960s works in (Carlile, 1970). Already in 1970s it was clear that nutritional value of a substance is not a prerequisite for its chemo-attractant effectiveness (Kincaid & Mansour, 1978, 1978a). Laboratory experiments confirmed that plasmodium is attracted to glucose, maltose, mannose and galactose (Carlile, 1970; Knowles & Carlile, 1978), peptones (Coman, 1940; Carlile, 1970), aminoacids phenylalanine, leucine, serine, asparagine, glycine, alanine, aspartate, glutamate, and threonine (Chet et al., 1977; Kincaid & Mansour, 1978; McClory & Coote, 1985). Molecular mechanisms and structure of carbohydrate recognition domain are proposed in (Kouno et al., 2011). Recently there was a re-ignition of interest to relation between chemo-attraction and their nutritional values (Dussutour, 2010). A plasmodium is allegedly indifferent to sucrose, fructose and ribose (Carlile, 1970; Knowles & Carlile, 1978), and repelled by sucrose and inorganic salts (Ueda et al., 1976; Adamatzky, 2010) and tryptophan (McClory & Coote, 1985). Some of the chemo-attractants may inhibit plasmodiums motion when the plasmodium comes into direct contact with such a substance. For example, galactose and mannose are reported to attract (Carlile, 1970; Knowles & Carlile, 1978) and also inhibit motion (Denbo & Miller, 1978) of *P. polycephalum*.

While constantly looking for novel ways to control propagation of slime mould, in order to program it and make it implement computational operations (Adamatzky, 2010a), we found (Adamatzky, 2011) that *P. polycephalum* is strongly attracted to herbal tablets Nytol[1], Kalms Sleep and Kalms Tablets[2]. The tablets are advertised as having the following sedation activities.

- Nytol: "herbal remedy to soothe and so aid restful sleep" (GlaxoSmithKlin, 2011)

[1]Distributed by GlaxoSmithKlin Consumer Healthcare, Brentford, TW8 9GS; manufactured by Brunel Healthcare Manufacturing Ltd, Swadlincote, DE11 0BB

[2]Marketed and manufactured by G.R. Lane Health Products Ltd, Gloucester GL2 0GR, UK

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TABLE 1. Active substances per tablet contained in Nytol, Kalms Tablets and Kalms Sleep (equivalent to mg of dry leaves or roots)

	Hops	Valerian Root	Passion Flower	Wild Lettuce	Vervain	Gentian	Total
Nytol	200	160	130	—	—	—	590
Kalms Sleep	30	180	90	90	30	—	440
Kalms Tablets	45	135	—	—	—	90	270

- Kalms Sleep: "blend of herbs which are traditionally used for their sedative action particularly in cases of sleep disorder. They are traditional remedy containing a blend of plant ingredients that help promote natural sleep" (G.R. Lane HealthProducts, 2011) .
- Kalms Tablets: "Kalms tablets are a traditional herbal remedy, which help relieve worry, irritability and the stress of everyday life. They also relieve symptoms of the menopause including worry, wakefulness, flushing and cold sweats." (G.R. Lane HealthProducts, 2011).

The sedation potential of the tables is due to their herbal contents, see Tab. 1:

- Valerian root (*Valeriana officinalis*): sedative properties are demonstrated in (Leathwood et al., 1982; Hendriks et al., 1985; Carlini, 2003; Houghton, 1999) systematic review of sleep-induced studies in (Bent et al., 2006); recent sleep inducing candidates of Valerian root are flavone glycoside linarin and valerenic acid (Fernandez et al., 2004); anti-anxiety and anti-depressant activity of valerian components contribute to sleep-enhancing properties (Hattesohl et al., 2008); anti-convulsive activity is shown in (Rezvani et al., 2010).
- Hops leaves (*Humulus lupulus*): sedative and anti-convulsion properties (Lee et al., 1993; Schellenberg et al., 2004; Hansel et al., 1980); anti-anxiety effects (Murphy et al., 2010).
- Passion flower (*Passiflora incarnate*): sedative and anti-anxiety (Dhawan et al., 2001; Soulimani et al., 1997); psycho-therapeutic (Zhang, 2004), anti-convulsive (Elsas et al., 2010), and sleep induction (Capasso & Sorrentino, 2005).
- Wild lettuce (*Lactuca virosa*): analgetic and sedative properties (Wesolowska et al., 2006; Sayyah et al., 2004);
- Gentian root (*Gentiana lutea*): anti-depressant activity (Haraguchi et al., 2004).
- Vervain (*Verbena officinalis*): cyto-protective effect on neurons of central nervous system (Lai et al., 2006) and analgesic (Calvo, 2006).

In experiments we calculate binary attractive preferences of *P. polycephalum* to the tablets and plants and construct a hierarchy based on degrees of chemo-attraction.

2. Experimental

We cultivate plasmodium of *P. polycephalum* in large plastic containers on slightly wet paper kitchen towels and fed it with rolled oats. For experiments we use round polystyrene Petri dishes 8 cm and 12 cm in diameter and 2% agar gel (Select Agar, Sigma Aldrich) as a non-nutrient substrate. Images of plasmodium are recorded by scanning Petri dishes in Epson Perfection 4490. We use three types of herbal sedation

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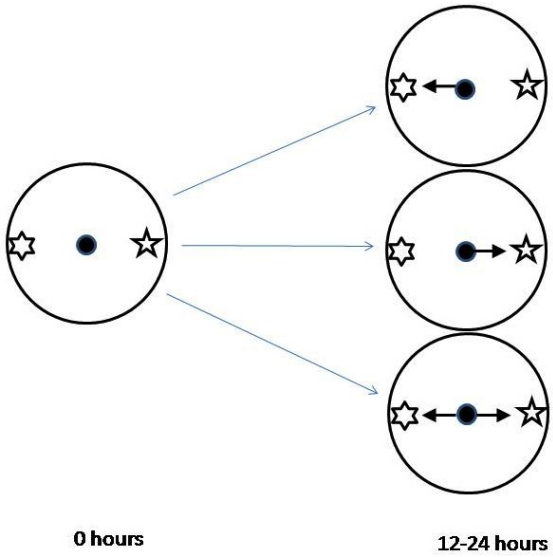

FIGURE 1. Experimental setup: locations of samples are shown by stars, inoculation site of plasmodium by solid disc. Propagation of plasmodium towards samples-targets is show by black solid arrows. Three scenarios are shown: plasmodium propagates towards sample marked by six-point star (top), plasmodium is attracted by sample marked by five-point star (middle), and plasmodium propagates simultaneously to both samples (bottom).

TABLE 2. Total number of experiments on binary choice conducted.

	K. Sleep	Valerian	Nytol	K. Tablets	Hops	P. Flower	W. Lettuce	Gentian	Vervain
K. Sleep	—	16	29	54	10	10	10	5	5
Valerian			33	14	21	7	12	6	12
Nytol				18	22	17	11	11	9
K. Tablets					5	5	8	11	9
Hops						9	5	5	10
P. Flower							5	10	5
W. Lettuce								5	5
Gentian									5

tablets Nytol, Kalms Sleep and Kalms Tablets. Active substances and other ingredients of the herbal tablets are shown in Tab. 1. We have chosen dry leaves and roots for further experiments of the following plants[3]: Valerian root (*Valeriana officinalis*), Hops leaves/buds (*Humulus lupulus*), Passion flower (*Passiflora incarnate*), Wild lettuce (*Lactusa verosa*), Gentian root (*Gentiana lutea*), Vervain (*Verbena officinalis*). Also in control experiments we used rolled oats[4], sugar[5] and honey[6].

[3]G. Baldwin & Co Ltd, London SE17 R1W

[4]Tesco Value Porridge Oats, Tesco Stores Ltd, Chestnut EN8 9SL

[5]Silver Spoon, Peterborough PE2 9AY

[6]Tesco Organic Squeezy Clear Honey, Tesco Stores Ltd, Chestnut EN8 9SL

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TABLE 3. Domination matrix D constructed on herbal tablets, valerian roots, honey and oats.

	K. Sleep	Valerian	Nytol	K. Tablets	Honey
K. Sleep	0	0.06	0.14	0.37	0.73
Valerian	0.88	0	0.52	0.79	0.86
Nytol	0.76	0.45	0	0.67	1
K. Tablets	0.41	0.21	0.22	0	0.71
Honey	0.13	0.07	0	0.19	0
Oat	0.14	0	0	0	0

TABLE 4. Domination matrix D constructed on tablets and plants.

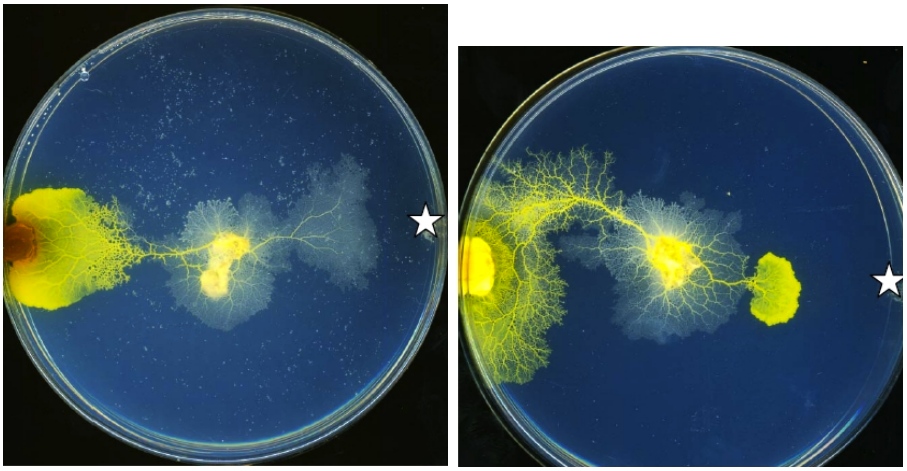
	K. Sleep	Valerian	Nytol	K. Tablets	Hops	P. Flower	W. Lettuce	Gentian
K. Sleep	0	0.06	0.14	0.37	0.7	0.7	0	1
Valerian	0.88	0	0.52	0.79	0.71	0.86	0.75	0.83
Nytol	0.76	0.45	0	0.67	0.36	0.65	0.83	0.9
K. Tablets	0.41	0.21	0.22	0	0.8	0.2	0.75	0.36
Hops	0.2	0.19	0.64	0.2	0	0.33	0.2	0.8
P. Flower	0.3	0.14	0.35	0.8	0.56	0	0.2	0.7
W. Lettuce	0.5	0.25	0.17	0.25	0.6	0.8	0	1
Gentian	0	0.17	0	0.36	0	0.3	0	0
Vervain	0.2	0.67	0.22	0.22	0.7	0	0	0.8

2.1. **Trials.** A scheme of a typical trial is shown in Fig. 1. We place an oat flake colonised by plasmodium, of *P. polycephalum* at the centre of a Petri dish, and a half of herbal tablet or 100 mg of dried herbs/roots at the opposite sides of the dish. Initially a plasmodium is positioned on a straight line connecting samples and at the same distance (65-70 mm) from the each sample. In 12-24 h from the inoculation the plasmodium propagates to one of the samples, or, rather infrequently, to both samples at once. Total number of experiments for each pair of samples is shown in Tab. 2: experiments for any given pair were stopped as soon as we observed convergence of plasmodium preferences to either sample of the pair.

2.2. **Domination.** Let **T**={ Nytol, Kalms Sleep, Kalms Tablets } be a set of tablets and **H**={ Valerian root, Hops, Passion flower, Wild lettuce, Gentian root, Vervain } be a set of dried herbs samples, and $\mathbf{S} = \mathbf{T} \cup \mathbf{H}$. For each pair of different samples $i, j \in \mathbf{S}$ we calculate a probabilistic domination matrix $D_{ij} = (E^i + E^j + E^{ij})/n$, where E^i and E^j are numbers of experiments where plasmodium chooses either sample i or j, and E^{ij} is a number of experiments where plasmodium propagates simultaneously to i and j, n is a total number of experimens. We use word domination in the following sense: sample i dominates sample j with probability D_{ij} if *P. polycephalum* prefers sample i to sample j with probability D_{ij}. Dominations matrices for sets of tablets and plants are shown in Tabs. 3 and 4.

3. RESULTS

Finding 1. *P. polycephalum prefers herbal sleep-remedy tablets (Nytol, Kalms Sleep and Kalms Tablets) to substances with higher ratio of carbohydrates (honey, oat flakes).*



(a) (b)

FIGURE 2. Herbal sleeping pills versus honey. (a) Kalms Sleep (left) vs Honey (right). (b) Kalms Tablets (left) vs honey (right). Position of honey drop is marked by star.

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To compare the tablets with honey and oat flakes we undertook at 8-16 experiments for each of the pair and calculated domination values. See example configurations in Fig. 2. Domination matrix is shown in Tab. 3. Plasmodium strongly prefers honey to oats (thus choosing sources with higher concentrations of carbohydrates) yet favours herbal tablets and valerian roots to oat or honey. In addition to herb extracts (Tab. 1) Kalms Sleep and Kalms Tablets contain sucrose (168 mg and 247 mg per tablet). Early studies (Carlile, 1970) demonstrated that sucrose does not attract plasmodium. Thus sucrose contents could not be kept responsible for the chemo-attraction properties of the tablets.

Finding 2. *P. polycephalum prefers Nytol to Kalms Sleep and Kalms Tablets while not showing any strong discrimination between Kalms Sleep and Kalms Tablets.*

When presented with a pair (Kalms Sleep vs. Kalms Tablets) plasmodium chooses Kalms Tablets with probability 0.41 and Kalms Sleep with probability 0.37 (Tab. 3). Slime mould prefers Nytol to Kalms Sleep with probability 0.76 and Nytol to Kalms Tables with 0.67. Thus higher degree of Nytols chemo-attraction is strongly pronounced. A mass of active substance may provide an explanation. There 1.3 times more active substance in Nytol than in Kalms Sleep, and over 2 times more in Nytol than in Kalms Tablets.

Finding 3. *P. Polycephalum prefers Valerian roots and Nytol to other dried herbs and herbal tablets. Kalms Sleep and Passion Flower, while less attractive than Nytol or valerian, stay at the top part of the preferences hierarchy, while Vervain, Hops and Gentian occupy lower part.*

See examples of patterns recorded in laboratory experiments in Fig. 3 and hierarchy of preferences, based on filtered domination matrix D (Tab. 4), in Fig. 4. From now on

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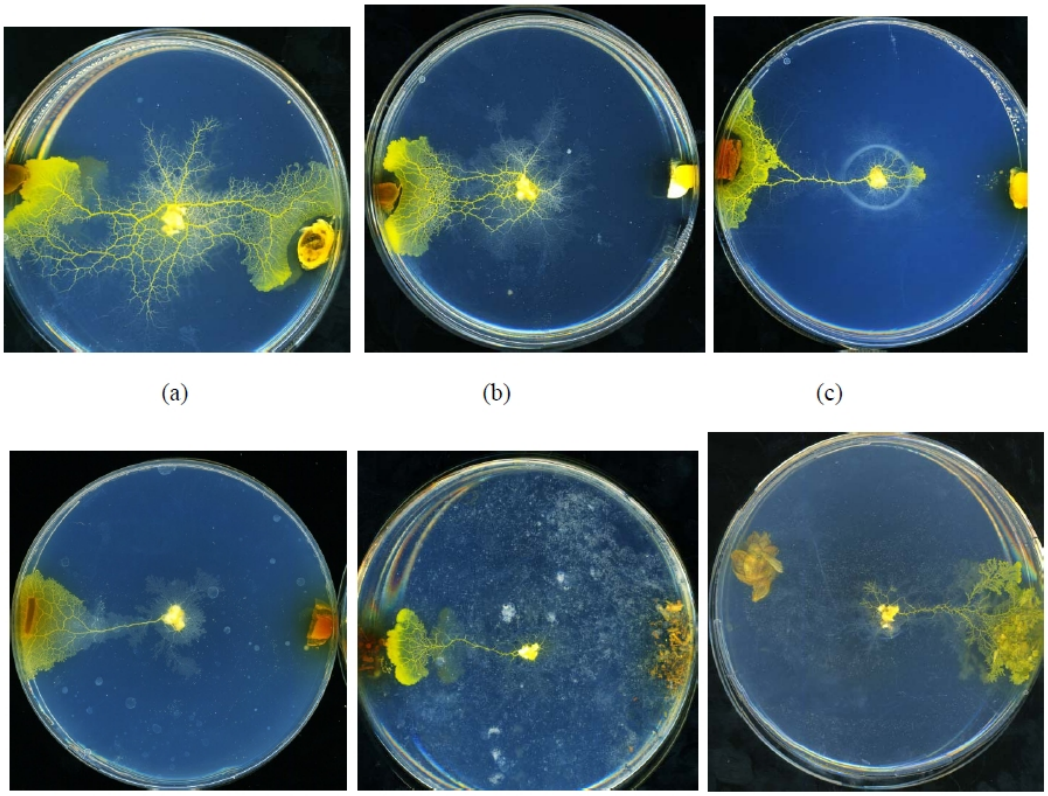

FIGURE 3. Examples of experiments. (ab) Kalms Sleep (left) vs Kalms Tablets (right): (a) plasmodium propagates to both targets, (b) plasmodium propagates to Kalms Sleep (left); (c) Nytol (left) vs Kalms Tablets (right); (d) Valerian roots (left) vs Nytol (right); (e) Valerian roots (left) vs. Vervain (right); (f) Hops (left) vs. Passion flower (right).

we are talking about strong domination: substance i dominates substance j if $D_{ij} > 0.7$. A strong hierarchy is an order of strong domination. Based on the strong hierarchy one may conclude that Valerian roots, Passion flower and Wild lettuce are the main components responsible for high degree of Nytols chemo-attraction.

Finding 4. *Herbal tablets obey the following hierarchy of domination*

$$\textit{Nytol} \rhd \{\textit{Kalms Sleep, Kalms Tablets}\}$$

while their hierarchy of domination recalculated via domination of their active components is

$$\textit{Nytol} \rhd \textit{Kalms Sleep} \rhd \textit{Kalms Tablets.}$$

Can we infer domination power of herbal tablets from weighted (i.e. multiplied by relative mass present in the tablet) domination powers of their active herbal components? Given set $\mathbf{A} = \mathbf{S}, \mathbf{T}$ or $\mathbf{H}$ we calculate domination of herbs on a set of herbs

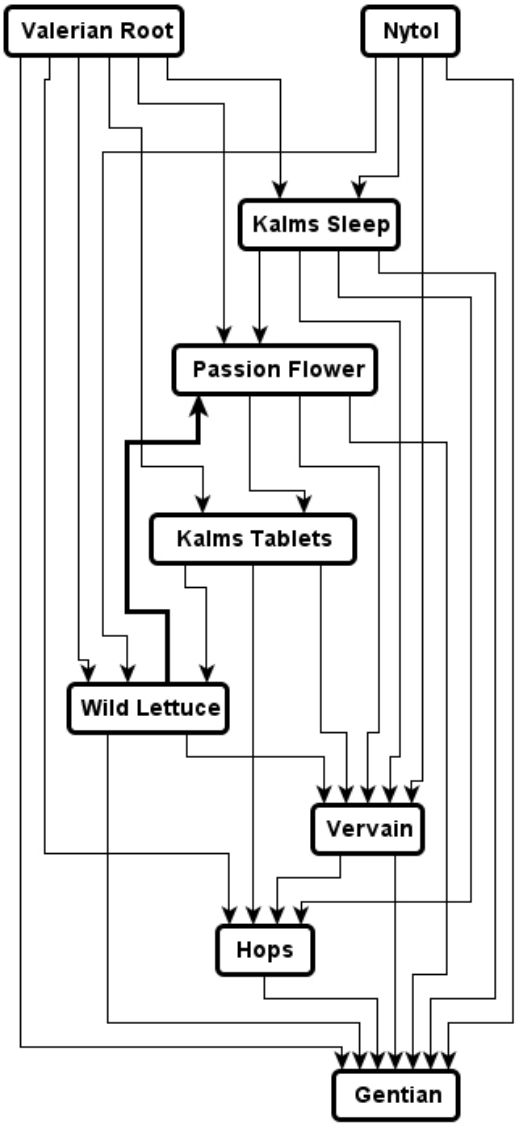

FIGURE 4. Hierarchy of strong domination. Arrow connects items i and j only if $D_{ij} > 0.7$. The higher is a substance positioned in the hierarchy the more strongly the substance attracts *P. polycephalum*.

as follows: $\Delta_i(\mathbf{A}) = \sum_{j \in \mathbf{A}} D_{ij}$. From Tab. 3 we see that $\Delta_{\text{Valerian Root}}(\mathbf{H}) = 3.49$, $\Delta_{\text{Hops}}(\mathbf{H}) = 1.83$, $\Delta_{\text{Passion Flower}}(\mathbf{H}) = 2.6$, $\Delta_{\text{Wild Lettuce}}(\mathbf{H}) = 3.65$, $\Delta_{\text{Gentian}}(\mathbf{H}) = 0.67$, and $\Delta_{\text{Vervain}}(\mathbf{H}) = 2.17$. Let c_{ij} be equivalent of dry herb i, measured in mg, contained in tablet j (Tab. 1). Let us calculate for each tablet j its domination W_j as a sum of weighted dominations of its active components (Tab. 4): $W_j = \sum_{i \in \mathbf{H}} \cdot 10^{-2} \cdot c_i^j \cdot \Delta_i(\mathbf{H})$: $W_{\text{Nytol}} = 13.1$, $W_{\text{Kalms Sleep}} = 12.4$, and $W_{\text{Kalms Tablets}} = 6.13$. These can be compared with dominations of tablets on the set of tablets $\Delta_{\text{Nytol}}(\mathbf{T}) = 1.43$, $\Delta_{\text{Kalms Sleep}}(\mathbf{T}) =$

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0.51 and $\Delta_{\text{Kalms Tablets}}(\mathbf{T}) = 0.63$ which is very close to domination of tablets on the set S of all samples: $\Delta_{\text{Nytol}}(\mathbf{S}) = 5.62$, $\Delta_{\text{Kalms Sleep}}(\mathbf{S}) = 3.74$, $\Delta_{\text{Kalms Tablets}}(\mathbf{S}) = 3.74$

We can speculate that some active components, particularly Passion flower, Wild lettuce and Vervain, do not work — as chemo-attractants for *P. polycephalum* — when extracted and combined in a pill as well as they do when presented to slime mould in a form of dried herbs.

Finding 5. *P. polycephalum preferences show transitive relations in all but Passion flower, Wild lettuce and Kalms Tablets subsets: slime mould prefers Passion flower to Calms Tablets and Calms Tablets to Wild lettuce but prefers Wild lettuce to Passion Flower.*

The 'offending' link is shown by bold in Fig. 4. Kalms Tablets do not contain Passion flower or Wild lettuce, therefore such a quasi-paradoxical cycle may be explained by presence of active substances and non-direct influence of their combination on chemo-attraction.

Finding 6. *Physarum follows gradients of airborne chemo-attractants.*

As far as we aware all previous studies on chemo-attraction of *P. polycephalum* were based on experimental setups where slime mould and chemo-attractants share the same substrate. Thus diffusion of active components in a substrate was considered the main vehicle of attraction. To demonstrate that it is not the case we undertook 15 probing experiments. In each experiment we placed samples of potential attractants on pieces of aluminium foil or plastic pads to prevent any direct contact of samples with agar gel. In all experiments plasmodium unequivocally detected locations of chemo-attractants. Examples in Fig. 5 demonstrate that plasmodium not only detects location of chemo-attractants but keeps order of its preferences even when met with several airborne gradients.

4. Discussion

We constructed hierarchy of chemo-attractive forces three types of herbal tablets and six species of plants with reported sedative, anti-convulsive and sleep-inducing properties. Valerian roots are the strongest chemo-attractant for *P. polycephalum.* Why is it so? Valerian contains hundreds of identified, and possibly the same amount of not yet identified, components including alkaloids (Torssell & Wahlberg, 1966), volatile oils (Hendriks & Bruins, 1980), valerinol (Jommi et al., 1967), and actinidine (Johnson & Wallera, 1971). Isovaleric acid and actinidine are identified in anal gland secretion of *Iridomyrmex nitidiceps* ant, and isovaleric acid is considered to be a distress indicator (Cavill et al., 1982). We can speculate that these components are also pheromones of *P. polycephalum* and could be considered in a framework of pheromones of cellular slime moulds (Lewis & O'Day, 1979; Newell, 1981; Nader & Shipley, 1984) (indeed, there may be pitfalls in projecting physiology of cellular mould to their acellular counterparts).

Actinidine is our main candidate on a role of strongest chemo-attractant for *P. polycephalum.* Future experiments should certainly be concerned with stimulating slime

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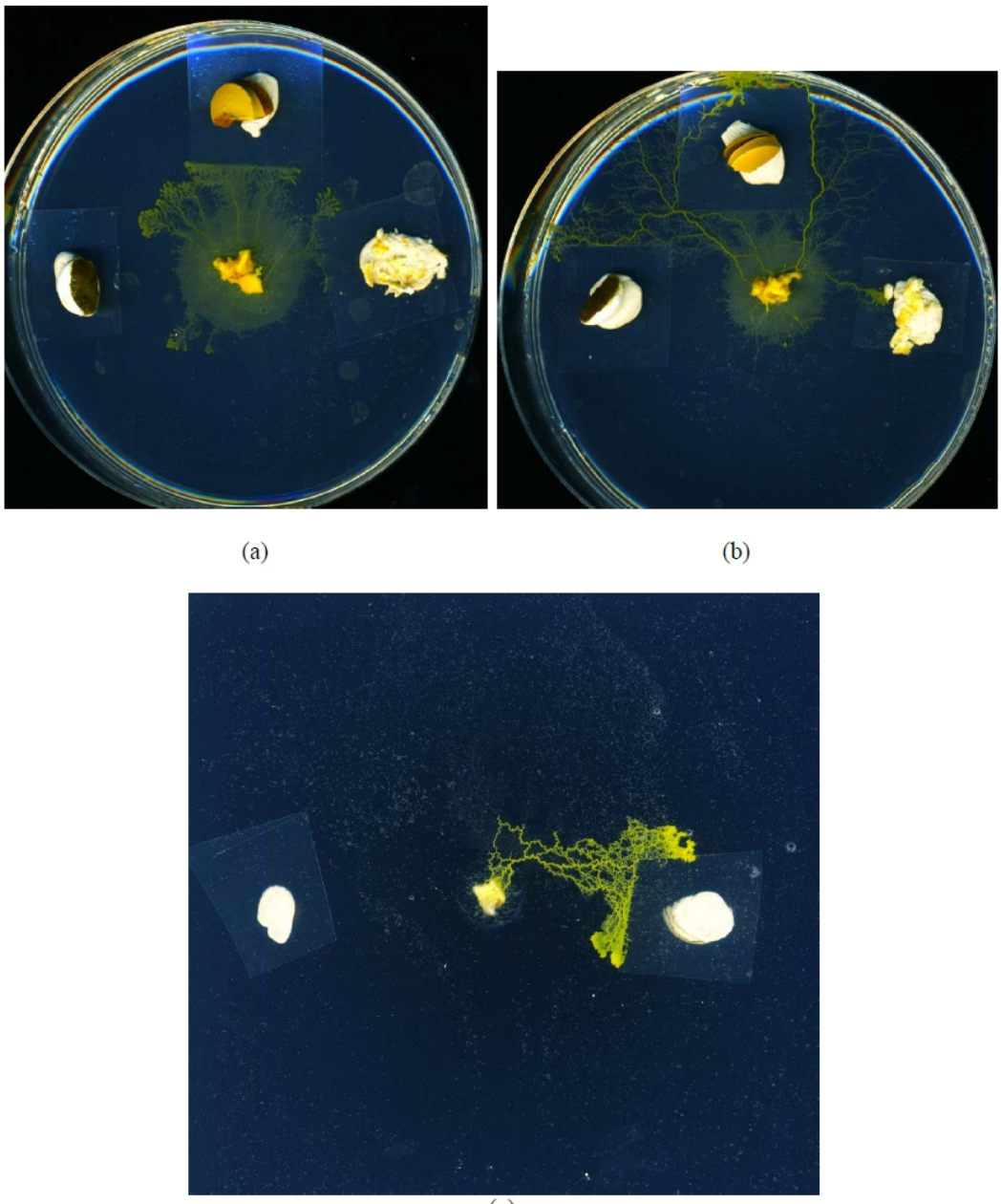

(a) (b)

(c)

FIGURE 5. Experiment on airborne attractants. Half-pills of Kalms Tablets (west), Nytol (north) and oat flakes (east) were attached by Blue-Tak to polyurethane pads placed on agar gel. An oat flake colonised by plasmodium is placed in the centre of Petri dish. (a) 8 hours from inoculation, (b) 18 hours after inoculation. (c) Plasmodium is challenged with Kalms Tablet (left) and Valerian roots (right) placed on plastic pads. Plasmodium moves towards Valerian roots.

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mould with artificially synthesised actinidine. We have done a set of experiments to collect rather circumstantial evidence. Actinidine is structurally close to terpenoid nepetalactone. Our assumption is that these substances attract cats, actinidine allegedly attracts slime mould, and therefore nepetalactone should be a chemo-attractant for *P. polycephalum*.

Nepetalactone is an active substance contained in Catnip Nepeta cataria. In 15 experiments we offered slime mould to choose between dried leaves of Catnip[7] and Valerian roots (sampled in 100 mg). In 11 experiments *P. polycephalum* preferred Valerian roots and it moved towards Catnip only in 4 experiments. This gives a preference of 0.73, which fits in our definition of the strong domination. Slime mould prefers Nytol to catnip in 6 out of 6 experiments. We suspect therefore that actinidine *per se* is not the main reason a slime mould is attracted to Valerian roots.

With regards to other parts of domination hierarchy (Fig.4) we believe a weak chemo-attraction force of hops, presented as dried herb sample, may be due to anti-microbial and anti-fungal activity of acids in hops (Simpson & Smith, 1992). Hops combined with Valerian, as in Nytol, Kalms Sleep and Kalms Tablets, show outstanding chemo-attraction. Combination of Hops and Valerian extracts reduces arousal caused by caffeine, allegedly via central adenosine mechanism (Schellenberg et al., 2004). We can speculate about a possible link between sedative activity and chemo-attraction as follows.

The plasmodium is a network of biochemical oscillators (Matsumoto et al., 1980; Nakagaki et al., 1999). Waves of excitation or contraction originate from several sources, e.g. induced by external stimuli and perturbations. The waves travel along the plasmodium and interact with one another in collisions. Growing and feeding plasmodium exhibits characteristic rhythmic contractions with articulated sources. The contraction waves are associated with waves of electrical potential change. We could propose that components of Valerian (likely actinidine in complexes with volatile oils) bind to receptors of plasmodium membrane and ultimately reduce amplitude and frequency of contractile oscillations (usually observed in plasmodial masses (Nakagaki et al., 2000)), cause relaxation of plasmodiums membrane. In the part of slime mould unaffected by active substances strength of oscillations remains unmodified. Thus cytoplasm is shifted towards relaxed parts and overall movement towards chemo-attractants is observed. Our analogies are speculative because analysis of receptor binding by Valerians components and studies of anti-spastic properties of valerian extracts have been done on mammals (Mennini, 1993; Marder et al., 2003; Abourashed et al., 2004; Circosta et al., 2007).

With regards to unconventional computing, we aimed to find a new range of active substances that could be used in programming and controlling behaviour of Physarum machines (Adamatzky, 2010a). This task is fulfilled. We determined several substances which offer a viable alternative to existing illumination- (Nakagaki et al., 1999), thermo- (Tso & Mansour, 1975) (Matsumoto et al., 1980) and salt-based repellents (Adamatzky, 2010), and classical carbohydrate-based attractants (Carlile, 1970) (Knowles & Carlile, 1978) (Dussutour, 2010).

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