

flowers: *Duranta repens* with nectarial tube 19–24%, *Jasminum angustifolium* 16–28%, and *Caesalpinia pulcherrima* 18–29%. But in species having bowl-shaped flowers, nectar sugar concentrations are high with glucose dominance. Earlier studies showed that nectars with higher sugar concentration (30–65%) are also intensively foraged by butterflies<sup>28,29</sup>, and the optimum concentration is approximately 40% (refs 28, 30). Nectar concentrations of *C. pyranthe* flowers (16–58%) stand close to the reported range of 15–50% in psychophilous flowers<sup>31</sup>. Flower visiting rate and flower handling time of *C. pyranthe* are comparable with those reported for pierid butterflies<sup>32</sup>. Although the pollination potentiality of a flower visitor is dependent on floral architecture<sup>33</sup>, deposition of pollen on mouthparts is considered to be important in psychophily<sup>31,34</sup>. Out of the 15 floral species tested in this context, *C. pyranthe* received pollen on the head in 14 species, proboscis 11, legs nine, antennae and wings six species each, thus qualifying itself as a pollinator of its floral species. It may be noted that a small proportion of butterfly pollination contributes to gene flow over relatively long distances<sup>34,35</sup>. This butterfly is an established migrant in India<sup>36</sup>, and migrant pollinators are receiving priority in the global conservation agenda<sup>37</sup>. Although Williams<sup>36</sup> indicated some directions in the movement of butterflies in India, there is an urgent need to trace the migratory corridor of *C. pyranthe* and identify ‘flowering waves’ along the corridor and determine the butterfly’s dependency on such ‘flowering waves’.

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## Phytochemical variability in commercial herbal products and preparations of *Withania somnifera* (Ashwagandha)

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*Withania somnifera* (Hindi – Ashwagandha, English – winter cherry) is used in Ayurvedic formulations for a variety of health-promoting effects. Several mono- and poly-herbal products commercially available in the Indian market were quantitatively analysed for a number of chemical constituents. The results revealed wide variations in the content of all seven constituents tested. More than 70-fold variation in the daily intake of withaferin A (the main active constituent of Ashwagandha) was found in the products. The study thus emphasizes the need for stringent phytochemical standardization of herbal products.

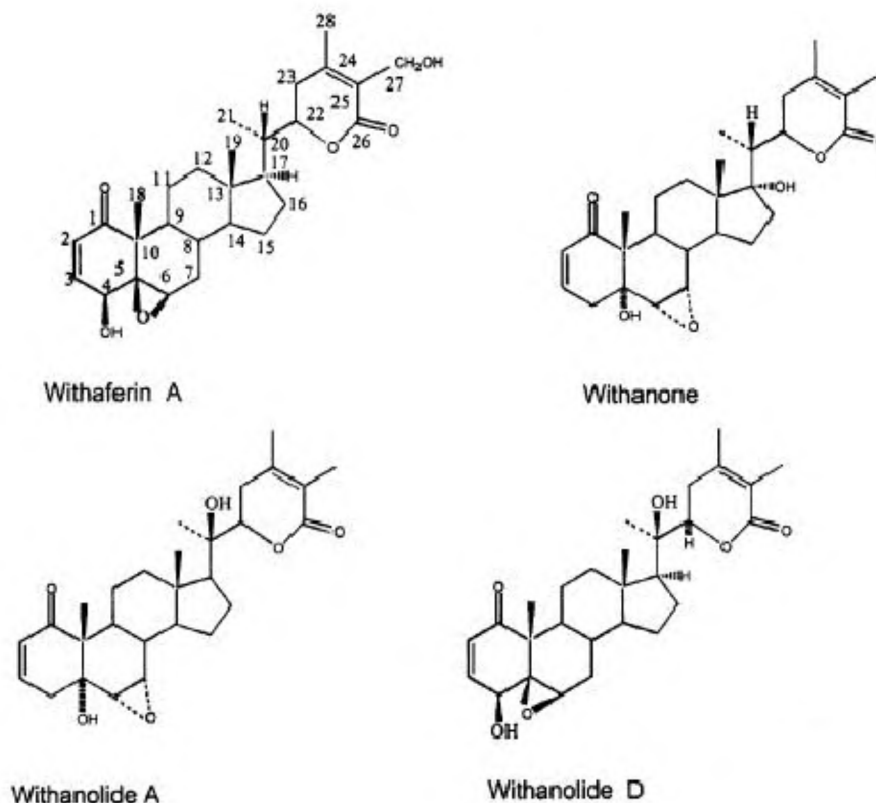
THERAPEUTIC properties of food and medicinal plants stem from the characteristic bioactive phytochemicals (mainly secondary metabolites) synthesized and amassed by them.

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*Withania somnifera* (Hindi – Ashwagandha, English – Indian ginseng/winter cherry) is one of the most valued medicinal plants in Ayurveda and other traditional systems of medicine. In Ayurveda, *W. somnifera* is regarded as one of the most useful herbs having 'Vata' pacifying properties<sup>1-3</sup>. In recent times, it has attracted tremendous pharmacological research interests. The plant has been reported to have adaptogenic, anticancer, anti-convulsant, immunomodulatory, antioxidative and neurological effects<sup>4-7</sup>. It is also considered efficacious in the treatment of arthritis, geriatric, behavioural and stress-related problems<sup>4-7</sup>. Withaferin A, chemically characterized as 4 $\beta$ ,27-dihydroxy-5 $\beta$ -6 $\beta$ -epoxy-1-oxowitha-2, 24-dienolide, is one of the main withanolidal active principles isolated from the plant (Figure 1). Withaferin A inhibits cyclooxygenase-2 (COX-2) but not cyclooxygenase-1 (COX-1)<sup>8</sup>, desired for a non-ulcerating anti-inflammatory/chemotherapeutic drug. Withaferin A has also been reported to have immunosuppressive action on B-lymphocyte proliferation<sup>9</sup>. Other withanolides, including glycosylated ones present in medicinal plants are reported to have antioxidant, immunomodulatory and other activities<sup>10-14</sup>. Some withanolides are known to have quinone reductase induction-mediated protective activity against chemical carcinogenesis<sup>15</sup>. A variety of mono- and poly-herbal preparations are commercially sold in India. However, no phytochemically

standardized pharmacoepial parameters are published for quality evaluation.

The present study was, therefore, undertaken to make an assessment of the phytochemical composition of commercial products of Ashwagandha. The amount of withaferin A and six other unidentified molecules was compared. The results show huge variations in the quantities of these chemical constituents in the products. The commercial products of Ashwagandha used in the present study are given in Table 1 along with their therapeutic and health-promoting properties detailed by the manufacturers. These products were procured from the market/generously provided by the manufacturers or their outlets and were coded as #001 to 010. The analyses are based on estimation of withaferin A and six other structurally unidentified phytochemicals designated as WS-1 ( $R_t$  8.9 min) WS-2 ( $R_t$  25.4 min), WS-3 ( $R_t$  25.7 min), WS-4 ( $R_t$  27.6 min), WS-5 ( $R_t$  29.3 min) and WS-6 ( $R_t$  45.1 min) with the help of HPLC analysis. The amount of individual constituent is expressed in mg in case of withaferin A or as peak area units (billion arbitrary units, bau) of the HPLC peak in a unit amount of Ashwagandha or in the suggested intake-dosage of the product per day. The study was intended only to display a general scenario of phytochemical variability in herbal/functional food products rather than their ranking or (dis)qualification.



**Figure 1.** Some withanolides of Ashwagandha (*Withania somnifera*).

**Table 1.** Ashwagandha products and their claimed therapeutic and health-promoting properties

Product	Manufacturer	Product type (Ashwagandha content)	Stated health benefits
Ashwagandharista	Baidynath Ayurved Bhawan	Polyherbal (1.70 g/30 ml)	Nervine tonic, memory and cognition improvement, better power of concentration, relieves mental tension, natural sleep induction, and recovery from nervous and general debility
Himalaya Ashwagandha	The Himalaya Drug Company	Monoherbal extract (250 mg/250 mg capsule)	Stress management
Stresswin	Baidynath Ayurved Bhawan	Polyherbal (200 mg/425 mg capsule)	Combating exertion, reduction in anxiety, strain and stress, improvement of stamina, relief from disturbed sleep, mental alertness, effective in convalescence, and relieves stress and strain during menopause
Stresscom	Dabur India Ltd	Monoherbal extract (300 mg/capsule)	Relieves anxiety neurosis, physical and mental stress, and relieves general debility and depression
Himalaya massage oil	The Himalaya Drug Company	Polyherbal (165 mg/ml)	Body relaxation, stress relief, and relief from insomnia, backache
Lovemax	BACFO Pharmaceuticals Ltd	Polyherbal (100 mg/500 mg capsule)	Vigour and vitality promotion
Vigomax	Charak Pharmaceuticals Pvt Ltd	Polyherbal plus Bhasma (extract from 300 mg w.s./capsule)	Vigour and vitality enhancement
Vital plus	Mukthi Pharma	Polyherbal (1.166 g/10 g)	Recovery from impotence, oligospermia, and recovery from general weakness, fatigue
Amrutha Kasthuri	Pankajakasthuri Herbals India Ltd	Polyherbal (2 g/15 g)	Convalescence, recuperation from general debility, neurasthenia, and better disease resistance
Brento	Zandu Pharmaceutical Works Ltd	Polyherbal (extract from 100 mg w.s./capsule)	Nervine tonic

The commercial products equivalent to 250 mg Ashwagandha (according to the label on the market sample) were taken. These were extracted (in case of solids) or mixed (in case of liquids) three times (5 ml each) with methanol. The extracts were added with 2.0 ml water and partitioned three times with equal volume of chloroform. The chloroform extracts were worked up and analysed by HPLC. Withaferin A, isolated from a plant accession (RS-1-CIMAP) of *W. somnifera* by chromatographic work-ups and structural analyses (IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, MS, etc.), served as reference. It was also used to develop a HPLC calibration curve for quantitative analyses. HPLC analysis was carried out on a reverse phase C-18 column (Waters) using methanol and water as mobile phase through a gradient elution of decreasing polarity under mild acidic conditions (details to be published elsewhere), with detection at 227 nm using a photo-diode array detector. For quantitative estimations, a calibration curve of withaferin A vs peak area and height in HPLC analysis was developed and the regression value (99% confidence) of the curve was used for quantitative computation of withaferin A in the samples.

The data are presented on the basis of amount of the phytochemicals per unit amount of Ashwagandha contained in the commercial products, range of variability (multiplicity factor with respect to the product with lowest content) and computed daily intake of the constituents according to suggested dose of the product. The product designation (001 to 010) in the analytical tables and description does not bear any correspondence with the sequence of listing of the products described in Table 1. Statistical analyses were carried out as descriptive statistics with the help of SPSS version 10.0 software.

**Table 2.** Withaferin A in selected commercial mono- and poly-herbal Ashwagandha products

Product	Withaferin A [mg g <sup>-1</sup> of Ashwagandha*]	Percentage of maximum in the sample	Withaferin A (mg) per suggested daily dose**	Dose multiplicity factor for Withaferin A
#001	2.34	100.0	1.40	70
#002	1.03	44.0	0.52	26
#003	0.93	39.7	0.37	18.5
#004	0.43	18.4	0.50–1.00	25–50
#005	0.43	18.4	0.86–1.72	43–86
#006	0.41	17.5	0.08–0.16	4.0–8.0
#007	0.32	13.7	0.06	3.0
#008	0.06	2.6	0.02	1.0
#009	0.05	2.1	n.a.	n.a.
#010	0.02	0.9	n.a.	n.a.

\*Ashwagandha state (herb or herb extract) as specified on the product.

\*\*Daily dose (load) of withaferin A computed from its concentration in the product and suggested intake of product.

\*\*\*The sequence of the herbal products mentioned here has no association with that in Table 1.

n.a., Not available/applicable.

ence of listing of the products described in Table 1. Statistical analyses were carried out as descriptive statistics with the help of SPSS version 10.0 software.

The amount of withaferin A estimated in some of the commercial brands of repute is given in Table 2. On a scale of 0 to 100%, the relative amount of withaferin A was nearly negligible, e.g. in products #008 to #010. This reflected a 117-fold higher concentration of withaferin A in the highest (product #001) holding product compared to one with the least (product #010). Daily dose of witha-

**Table 3.** Content of unidentified Ashwagandha-specific phytochemicals in selected commercial mono- and poly-herbal products. Indications in parentheses are the HPLC chromatogram retention times of the phytochemicals under the conditions of analysis

Product	Amount of phytochemicals identified according to HPLC retention time (billion arbitrary units per g of Ashwagandha in the product)					
	WS-1 (8.9 min)	WS-2 (25.4 min)	WS-3 (25.7 min)	WS-4 (27.6 min)	WS-5 (29.3 min)	WS-6 (45.1 min)
#001	1.41	0.58	n.d.	n.d.	0.38	1.22
#002	0.21	0.36	0.30	0.02	0.32	0.92
#003	3.10	n.d.	0.68	0.06	0.38	1.14
#004	0.13	0.08	n.d.	n.d.	0.05	0.17
#005	n.d.	0.67	n.d.	n.d.	0.23	0.55
#006	9.18	n.d.	0.45	0.94	0.78	0.17
#007	n.d.	n.d.	n.d.	1.24	n.d.	0.30
#008	27.8	0.03	n.d.	0.11	n.d.	n.d.
#009	4.94	n.d.	0.05	0.79	n.d.	n.d.
#010	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

\*Ashwagandha state (herb or herb extract) as specified on the product.  
n.d., Not detected.

ferin A (according to recommended intake of the product) ranged from as low as 0.02 mg to as high as 1.4 mg. Data in Table 3 suggest that some of the products had widely varying relative values (considering the highest content as 100%) of each of the six constituents. For example, the minimum content (relative to 100% in the product with maximal content) was represented by only 1% in case of WS-1 (#002 and #004), less than 5% in case of WS-2 (#008), less than 10% in case of WS-3 (#009), less than 5% in case of WS-4 (#002 and #003), less than 10% in case of WS-5 (#004), and less than 15% in case of WS-6 (#004 and #006). In terms of the recommended daily dose of the product, intake varied in excess of 80-fold for WS-1, 260-fold for WS-2, up to three fold for WS-3, 38-fold for WS-4, 15-fold for WS-5 and 36-fold for WS-6. Statistical analysis of the daily dose of active phytochemicals from the suggested daily ingestion of the product gave extremely high dispersion values (variance and standard error) around the mean values. The results clearly suggest that the commercial products vary by several orders of magnitude with respect to their phytochemical composition. Since phytochemicals, particularly unique ones (like withanolides in Ashwagandha) are *raison d'être* for health benefits of the herbs, inordinate variations in their daily dose may reverberate in blotchy health benefit(s), or convolute development of quantifiable/testable pharmacopoeia for the products. Even if some/several of the constituents are not related to therapeutic benefit(s), assumption of the 'null effect' of their hypo- or hyper-doses may be resisted.

These variations can emanate from several factors that include: (i) diversified bioresources of heterogenous nature from the wild and/or under cultivation, (ii) physiological and ecological variations in plantations, (iii) harvest and post-harvest operations, (iv) processing of biomass, (v) manufacture process for product, (vi) unregulated and often non-descript supplementations, etc. In Israel and South

Africa, three major chemotypes have been reported in *W. somnifera*<sup>2,6</sup>. Chemotype variation of *W. somnifera* has not been studied in India. Our studies are in progress on Ashwagandha lines to develop chemotypes/chemovars under NMITLI (New Millennium Indian Technology Leadership Initiative). Efforts towards narrowing down the phytochemical variations and maintenance of compositional uniformity of Ashwagandha-based herbal products are also necessitated in view of tightening regulatory frameworks<sup>16-19</sup> like Dietary Supplements and Health Education Act and the new Natural Health Product Regulations 2003.

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## Female remating in *Drosophila*: Comparison of duration of copulation between first and second matings in six species

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**Female remating is the fundamental to evolutionary biology, as it determines the pattern of sexual selection and sexual conflict. During the course of the present study, female remating behaviour of six species of *Drosophila*, *D. ananassae*, *D. nasuta*, *D. eugracilis*, *D. melanogaster*, *D. simulans* and *D. pseudoananassae* was observed and compared. Periodic confinement**

**design (2 h daily observation) was used for female remating experiments. The frequency of female remating ranges from 16 (*D. eugracilis*) to 82% (*D. melanogaster*) in different species of *Drosophila*, and the differences among different species are statistically significant. Remating latency also varies from 5.51 days (*D. melanogaster*) to 9.85 days (*D. pseudoananassae*) in different species, and variation among different species is statistically significant. Duration of copulation in first (virgin mating) and second (remating) matings was observed and compared in each of the six species. Among all the species tested, females of *D. ananassae*, *D. nasuta*, *D. eugracilis*, and *D. pseudoananassae* show significantly shorter duration of copulation in the second mating compared to the first mating, while *D. simulans* females show shorter duration of copulation in the second mating compared to the first mating, but the difference is statistically not significant. However, *D. melanogaster* females show significantly longer duration of copulation in the second mating compared to first mating. Based on these findings, it may be suggested that different species of *Drosophila* may vary in the incidence of remating and duration of copulation due to differences in their reproductive biology and adaptation.**

MATING by animals is an important component of sexual behaviour, and transfer of sperm to females is the primary function of mating in sexually reproducing animals. Since each mating provides an opportunity to produce offspring, males can generally increase their fitness by mating with many females<sup>1</sup>. However, females intensify their reproductive success by increasing the number of viable eggs produced. This basic asymmetry between the sexes results in sexual conflict over remating, which suggests that male fitness increases monotonically with increased mating rate, while single or a few matings are sufficient for females to maximize their reproductive success. However, females of a majority of animal species mate several times, most often with different males (polyandry–multiple mating or remating), but also with the same male (repeated mating)<sup>2</sup>.

Female remating is an important component of *Drosophila* mating systems because after mating, the females store a large number of sperms in the paired spherical spermathecae and a single elongate tubular seminal receptacle<sup>3</sup> and utilize them to fertilize eggs as they are laid. Once a virgin female *Drosophila* has mated, she is usually unwilling to accept another male for some time because after mating, behavioural and physiological changes occur, including decrease in attractiveness to males<sup>4</sup>, decreased receptivity to further mating<sup>5</sup>, increasing of oogenesis, ovulation and oviposition rates<sup>4</sup>, storage and utilization of sperm<sup>6</sup> and decreased lifespan<sup>7</sup>. These behavioural and physiological alterations after mating in females have both short and long-term effects. The short-term effect, also called the ‘copulation effect’ in *Drosophila* is due to seminal fluid components transferred during mating by males, which cause the initial decrease in receptivity<sup>4</sup>, thus maximizing sperm usage and minimizing the chance

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