fluenced by an underlying bias in dinucleotide usage, for example, genes located in GC-rich regions of the chromosome preferentially utilize GC ending codons.

It is important for heterologous gene expression to encode proteins with sequences that yield optimal expression. A good thumb rule for finding such an optimal sequence is to choose codons that are most frequent in highly expressed genes. The CAI provides an explicit way of finding such codons; the most frequent codons simply have highest relative adaptiveness values, and sequences with higher CAIs are preferred over those with lower CAIs.

The study gives comprehensive information regarding the CAI and GC content of RNA genome plant viruses, and its influence on amino acid content.


1. Sharp, P. M., Stenico, M., Peden, J. F. and Lyod, A. T., *Biochem. Soc. Trans.*, 1993, **21**, 835–841.
2. Lobry, J. R. and Sueoka, N., *Genome Biol.*, 2002, **3**, 58.
3. Chen, S. L., Lee, W., Hottes, A. K., Shapiro, L. and McAdams, H. H., *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 3480–3485.
4. Vartanian, J. P., Henry, M. and Wein-Hobson, S., *J. Gen. Virol.*, 2002, **83**, 801–805.
5. Aota, S. and Ikemura, T., *Nucleic Acids Res.*, 1986, **14**, 6345–6355.
6. Francino, M. P. and Ochman, H., *Nature*, 1999, **400**, 30–31.
7. Kanaya, S., Yamada, Y., Kinouchi, M., Kudo, Y. and Ikemura, T., *J. Mol. Evol.*, 2001, **53**, 290–298.
8. Haas, J., Park, E. C. and Seed, B., *Curr. Biol.*, 1996, **6**, 315–324.
9. Jansen, R., Bussemaker, H. J. and Gerstein, M., *Nucleic Acids Res.*, 2003, **31**, 2242–2251.
10. Mooers, A. O. and Holmes, E. C., *Trends Ecol. Evol.*, 2000, **15**, 365–369.
11. Wolfe, K., Sharp, P. M. and Li, W. H., *Nature*, 1989, **337**, 283–285.
12. Sharp, P. M. and Li, W. H., *Nucleic Acids Res.*, 1987, **15**, 1281–1295.
13. Drake, J. W. and Holland, J. J., *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 13910–13913.


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# Antibacterial principles from the bark of *Terminalia arjuna*

The arjun tree *Terminalia arjuna* (Roxb.) is a well-known medicinal plant whose bark is extensively used in ayurvedic medicine, particularly as cardiac tonic. The bark is also prescribed in biliousness and sores and as an antidote to poison, and it is believed to have an ability to cure hepatic, congenital, venereal and viral diseases. A decoction of its bark with cane sugar and boiled cow's milk is highly recommended for endocarditis, pericarditis and angina[1].

Infectious endocarditis is an inflammatory disease of the endocardium, the internal lining of the human heart caused by bacteria such as staphylococci and gonococci. Among staphylococci, *Staphylococcus epidermidis* is one of the major etiological agents of this disease. The infections occur mainly in patients with prosthetic heart valves and during simple hospital procedures like catheterization, insertion of intra-uterine contraceptive devices, intravenous injections, etc.

In our screening programme aimed at detecting biomolecules from plant sources, which can specifically act against *S. epidermidis*, we found that the bark extracts of *T. arjuna* possessed antibacterial activity. Bioactivity-directed fractionation of the active extracts yielded three known oleane compounds: arjunic acid (**1**), arjungenin (**2**), and arjunetin (**3**), which were found to possess activity against *S. epidermidis*. The results presented here validate the traditional use of bark extracts of *T. arjuna* to cure endocarditis.

The bark of *T. arjuna* was collected from the CIMAP medicinal plants conservatory, during January 1999, identified in the Department of Botany and Pharmacognosy at CIMAP, where a voucher specimen (no. 5867) is maintained. The air-dried, powdered bark material was successively extracted with hexane and ethanol to yield hexane-soluble and alcohol-soluble fractions. The hexane and ethanol-insoluble plant material was extracted in water to get the water-soluble fraction. The alcohol-soluble extract was subsequently extracted with diethyl ether, ethyl acetate and methanol to yield the corresponding extracts.

For the isolation of pure molecules, *T. arjuna* (4.5 kg) was air-dried, crushed, powdered and extracted with hexane ($3 \times 5$ l) at room temperature to remove fatty materials. The material was extracted with ethanol ($3 \times 5$ l). The combined extract was concentrated under vacuum and further extracted using diethyl ether, which afforded 152 g of diethyl ether-soluble extract. The diethyl ether-soluble portion was column chromatographed over silica gel (60–120 mesh, 1200 g) using varying proportion of hexane : ethyl acetate (98 : 2, 95 : 5, 90 : 10, 85 : 15, 80 : 20, 75 : 25, 70 : 30, 60 : 40, 50 : 50, 40 : 60, 30 : 70, 20 : 80, 10 : 90, 100 : 0) as eluent. 100 ml of each fraction was collected and monitored by TLC.

Fraction nos 325–442 afforded compound **1**, identified as arjunic acid on the basis of spectral analysis[2–5], using hexane–ethyl acetate as eluent in the ratio (50 : 50 v : v) and crystallized using methanol.

Fraction nos 538–800 afforded compound **2**, identified as arjungenin by spectral analysis[3,6], using hexane–ethyl acetate as eluent in the ratio (50 : 50 v : v) and crystallized using methanol.

Fraction nos 949–1377 afforded compound **3**, identified as arjunetin by spectral analysis[3,7], using hexane–ethyl acetate as eluent in the ratio (20 : 80 v : v) and crystallized using methanol.

The bacterial sensitivity testing was done on the Mueller–Hinton (Hi-Media) agar No. 2. All the bacterial strains were grown on nutrient agar/broth for routine cultivation. *Streptococcus mutans* was grown on brain–heart infusion agar at 28°C. Tetracycline was used as control antibiotic in the bioactivity assays with bacteria. The test compounds were dissolved in DMSO and stored at 4°C until use.

Preliminary antibacterial activity testing was done according to Bauer *et al.*[8]. All bacteria were sub-cultured from –80°C stock cultures into 5 ml of Mueller–Hinton broth and incubated for 24 h at the desired temperature. For use as an inoculum, the turbidity of the bacterial suspension was adjusted to the McFarland standard 0.5 (ca. $1.5 \times 10^8$ cfu/ml). About 100 µl of bacterial culture was spread-plated on solid medium and discs (5 mm diameter) containing test compound were placed on the pre-inoculated agar surface. Observations were recorded after 48 h of incubation of plates at the desired temperature.

Twofold serial dilution technique was employed to assess the minimum inhibitory concentration (MIC) of a given compound using 96-well microplate. Optical density of the cultures was measured using Spectra Max 190 microplate reader (Molecular Devices Corp. USA). MIC was taken as the lowest concentration of the test compound which inhibited the appearance of visible growth.

**Table 1.** Minimal inhibitory concentration of tri-terpenoid compounds isolated from diethyl ether fraction of *Terminalia arjuna* extract

| Compound | Minimal inhibitory concentration (µg/ml) | | | |
|---|---|---|---|---|
| | *Sreptococcus mutans* | *Staphylococcus epidermidis* | *S. aureus* | *Pseudomonas aeruginosa* |
| Arjunic acid (**1**) | 250 | 250 | >1000 | >1000 |
| Arjungenin (**2**) | 500 | 500 | >1000 | >1000 |
| Arjunetin (**3**) | >500 | **125** | >500 | >500 |

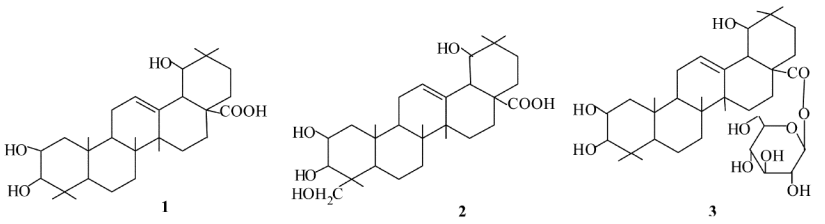


**Figure 1.** Chemical structure of triterpenes isolated from the bark of *Terminalia arjuna*: arjunic acid (**1**), arjungenin (**2**) and arjunetin (**3**).

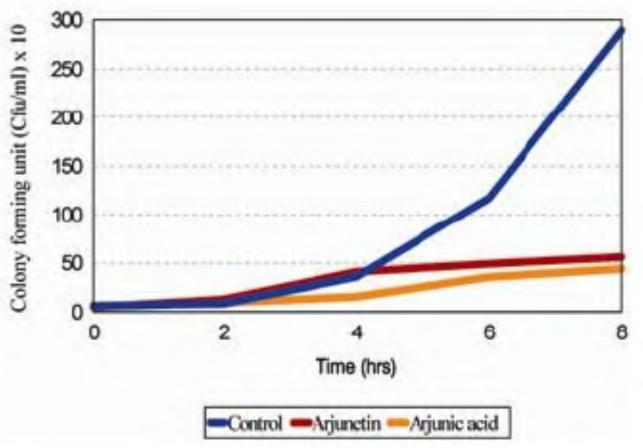


**Figure 2.** Effect of arjunetin and arjunic acid on the growth of *Staphylococcus epidermidis*.

Bioassays indicated that all the extracts except the hexane fraction possessed significant activity against Gram-positive bacteria such as *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Streptococcus mutans* (MTCC 890), *Bacillus subtilis* (MTCC 121) and *Mycobacterium smegmatis* MC$^2$ 155 (provided by Dr Anil K. Tyagi, UDSC). *S. mutans* and *S. epidermidis* were particularly highly sensitive to all the extracts. The initial crude bark extract also exhibited activity against *Klebsiella pneumoniae* (MTCC 109) and *Enterococcus faecalis* (MTCC 439), which however could not be detected in any of the solvent fractions. In general, the crude extract and the solvent fractions were inactive against Gram-negative bacteria. The water-insoluble substances have to be absorbed through the rather thick impermeable lipopolysaccharide layer, which presents a physical barrier in Gram-negative bacteria. Hence, the inactivity of extracts against Gram-bacteria is understandable. Thus, only small terpenoids which are able to form hydrogen bonds and therefore significantly interact with water, may be better able to traverse these pathways[9].

The diethyl ether fraction was further taken up for bioactivity-guided fractionation using silica gel column chromatography. Three pure tri-terpenoid compounds, viz. arjunic acid, arjungenin, arjunetin (a glycoside derivative of arjunic acid), the structures of which were confirmed by spectral analysis (Figure 1), were isolated. Arjunic acid and its glycosilated derivative showed moderate activity against *S. epidermidis* with MIC of 125 µg/ml (Table 1). The glycosylation of arjunic acid increased its activity twofold against *S. epidermidis*. Growth of this bacterium in the presence of arjunetin and arjunic acid at twofold lower concentration than that of the MIC (i.e. 62.5 and 125 µg/ml) indicated that there was no reduction in the initial titre even after 8 h (Figure 2). Hence the compounds could be bacteriostatic in nature. These findings indicate the presence of antibacterial principles in the bark of *T. arjuna* with arjunetin particularly showing selectively higher activity against *S. epidermidis*. Further derivatization of this molecule through structure–activity relationship studies

may lead to development of new antibiotic(s) of high potency.

1. Kumar, D. S. and Prabhakar, Y. S., *J. Ethanopharmacol.*, 1987, **20**, 173–190.
2. Row, L. R., Murty, P. S., Subba Rao, G. S. R., Sastry, C. S. P. and Rao, K. V. J., *Indian J. Chem.*, 1970, **8**, 716–721.
3. Honda, T., Murae, T., Tsuyuki, T., Takahashi, T. and Sawai, M., *Bull. Chem. Soc. Jpn.*, 1976, **49**, 3213–3218.
4. Anjaneyulu, A. S. R. and Rama Prasad, A. V., *Phytochemistry*, 1982, **21**, 2057–2060.
5. Honda, T., Murae, T., Tsuyuki, T. and Takahashi, T., *Chem. Pharm. Bull.*, 1976, **24**, 178–180.
6. Anjaneyulu, A. S. R. and Rama Prasad, A. V., *Indian J. Chem. B*, 1982, **21**, 530–533.
7. Tsuyuki, T., Hamada, Y., Honda, T., Takahashi, T. and Matsushita, K., *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3127–3128.
8. Bauer, A. W., Kirby, W. M. M., Sherries, J. C. and Turck, M., *Am. J. Clin. Pathol.*, 1966, **45**, 493–496.
9. Griffin, S. G., Wyllie, S. G., Markham, J. L. and Leach, D. N., *Flavour Fragrance J.*, 1999, **14**, 322–332.

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# Indian Ocean dipole mode and tropical cyclone frequency

The Indian Ocean dipole[1] is a coupled ocean–atmosphere phenomenon observed in the Indian Ocean (IO) in the form of an east-west dipole in the sea surface temperature (SST) anomalies. The Indian Ocean Dipole Mode Index (IODMI) is defined as the difference in SST anomaly between the tropical western IO (50–70°E, 10°S–10°N) and the tropical southeastern IO (90–110°E, 10°S-equator)[2]. Positive IODMI is associated with warm SST anomaly over the western tropical IO and cold SST anomaly over the southeastern tropical IO. Sign of the index reverses when SST anomalies swing to the opposite phase. The IODM phenomenon seems to play a key role in the occurrence of droughts (Tuarang) over the Indonesian region[3–5]. When IODMI is negative, it leads to drought over Indonesia and floods over East Africa and vice versa. Positive IODMI seems to correspond to more monsoon rains over India. The northeast monsoon rainfall (October–December) over south peninsular India and the IODM (September–November) are directly related, suggesting that the positive (negative) phase of the mode enhances (suppresses) the northeast monsoon activity[6]. A recent study has shown that the Indian summer monsoon has greater influence on the IODM, than vice versa[7]. In the present work, the relationship between the IODM and cyclone frequency in the Bay of Bengal (BOB) during the post-monsoon season (October–December), which is also known as the storm season in South Asia, has been investigated. The probable impact of IODM on the frequency of monsoon depressions and storms (which are significant rainfall-producing systems during the monsoon season from June to September) has also been looked into. Monthly time-series of IODMI[8,9] from January 1958 to December 2002 has been used to determine the correlation with cyclone frequencies. The cyclone frequency data have been obtained from IMD records.

As mentioned earlier, the post-monsoon season from October to December is known for maximum number of intense cyclones in the north IO[10]. Monthly frequency of severe cyclones (with maximum sustained wind speed exceeding 48 nmile per h) is highest during November. As about 80% of the north IO tropical cyclones form in the BOB, the focus of the present work is on the relationship between the IODM and the tropical cyclone frequency in the BOB.

The time-series of IODMI during September–October and the cyclone frequency in the BOB during November are presented in Figure 1. The aim is to demonstrate the lag relationships which have forecasting applications. It is evident from Figure 1 that the IODMI of preceding two months has an inverse relationship with the cyclone frequency in November. In other words, the negative IODMI during September–October corresponds to enhanced cyclone frequency during November. Therefore, colder SST anomalies over the western tropical IO and warmer SST anomalies over the southeastern tropical IO during September–October will correspond to enhanced cyclone activity in the BOB during November.

Figure 2 depicts the correlation coefficient (CC) between IODMI (September–October) and cyclone frequency (November). The CC between the September–October IODMI and November cyclone frequency is about –0.4, which is statistically significant at the 99% level. Thus the IODMI can provide useful indications of the November cyclone frequency two months in advance, which could be a potential tool in tropical cyclone forecasting. It is interesting to note that the simultaneous correlation between the November IODMI and the November cyclone frequency is less (–0.34) than the lag correlations, which implies that SST anomalies during the preceding two months play a more important role in the cyclogenesis in the BOB during November, than the anomalies during November.

The inverse relationship between the IODMI and the November cyclone frequency is consistent with the circulation patterns associated with the extreme dipole events during the post-monsoon season. A notable feature observed during the positive (negative) phase of the dipole mode is an anomalous anticyclonic (cyclonic) circulation over the BOB[6]. Thus the anomalous cyclonic circulation during the negative phase may support the formation of cyclones over the BOB as the anomalous cyclonic vorticity triggers the genesis of cyclones. This is the possible reason for the nega-