# Science Last Fortnight

### *Sowa Rigpa* in Bhutan

*Mining medicinal minerals*

In *Sowa Rigpa*, herbs, animal parts, and minerals are used as raw materials for medicine. Though research has identified and authenticated many plant and animal parts, investigations on mineral components of *Sowa Rigpa* are scarce.

Scientists from India, Bhutan, USA, Germany and Australia collaborated to address the issue. They tackled the problem of chemically identifying various minerals and their medicinal use in the Bhutanese medicine system.

Douglas J. McLaughlin, via Wikimedia Commons

The researchers first surveyed available traditional medical literature. They reviewed and evaluated *Sowa Rigpa* textbooks and other documents to generate a list of minerals and their uses.

Then, they made a list of mineral ingredients used in current formulations at the Menjong Sorig Pharmaceuticals, Bhutan. This company produces more than one hundred formulations, distributed for health care and treatment in Bhutan.

Next, the team cross-checked the chemical names and composition of the identified minerals against mineral databases and handbooks. Then, they authenticated and standardised *Sowa Rigpa* names through open-forum discussions with Bhutanese government health officials, traditional medicine practitioners and an expert from the Menjong Sorig Pharmaceuticals.

In this manner, the researchers identified about a hundred and twenty minerals described in the *Sowa Rigpa*, out of which about thirty are currently used in multi-ingredient prescription medicines in Bhutan. The company has more than a hundred formulations that use these minerals to treat several ailments. Six of these mineral ingredients are precious metals and stones. The rest include earth, mud, stones, salts, essences and exudates.

These findings can help source mineral ingredients from Bhutan, reducing costs, and generating income for farmers and miners there, say the scientists. The study also opens up the need to assess the efficacies and to investigate the potential uses of other minerals listed in the *Sowa Rigpa*. The medical system is an accepted part of the AYUSH programme in India where such research can be taken up in an interdisciplinary manner.

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### Weighing Olanzapine Use

*CART carries solution?*

People diagnosed as psychotic are prescribed olanzapine. However, taking the drug makes one eat a lot and gain weight. Though we know that it influences many neurotransmitters and neurosteroids, we do not know what makes those who take olanzapine voracious eaters.

When researchers were looking at weight loss in those who misuse amphetamines and cocaine, they found an increased quantity of a transcript in the hypothalamus, the part of the brain that controls food intake and energy homeostasis. They called it the Cocaine and Amphetamine Regulated Transcript or CART for short.

Dadasaheb Kokare from the Rashtrasant Tukadoji Maharaj Nagpur University and team hypothesised that the peptide, CART, is perhaps, involved in making people on olanzapine overeat. Dadasaheb teamed up with Nishikant Subhedar, IISER Pune, and Kartik Nakhate, Rungta College of Pharmaceutical Sciences and Research, Chhattisgarh to test the hypothesis on rats.

They gave olanzapine to rats every day for fourteen days, and monitored food intake and body weight gain. As expected, the rats became voracious, eating about 50% more than controls. They started gaining weight by the third day and, by the fourteenth, they were 14% heavier on an average, than controls.

The researchers examined the brains of the rats, using immunofluorescence to check for CART. The method is simple in principle. Raise antibodies against CART, and attach the antibodies to fluorescent material. The antibody will bind to CART in the brain tissue and, because of the fluorescence, we can see where CART is located.

When Subhedar and team looked at the brains of rats on olanzapine, they found a reduction in CART in the hypothalamus.

Can overeating and weight gain be countered by increasing CART? Subhedar and team pretreated another set of rats with CART and then gave them olanzapine. These rats did not gain weight.

This suggests that CART is responsible for weight gain in people who take olanzapine.

'These findings may help target alternative pathways to regulate energy balance to address the negative clinical effects of olanzapine', says Subhedar.

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### Increase longevity!

*Juniper berries*

By MPF, via Wikimedia Commons

The female seed cones of *Juniperus communis* L., a conifer found in the Western Himalayas from Kashmir to Kumaon, are used in a variety of ayurvedic medicines. Recent scientific investigations have found that the secondary metabolites of this

shrub have antioxidant and antimicrobial properties. However, most such studies were *in vitro* and needed follow up with *in vivo* studies.

Recently, scientists from the CSIR-National Botanical Research Institute, Lucknow, investigated the *in vivo* antioxidant and longevity-promoting effect of essential oil from the juniper berry.

The research team used the free-living nematode, *Caenorhabditis elegans*, a model organism. Because of its short life cycle of up to 20 days, it is well suited for ageing studies.

The team tested different concentrations – 0, 10, 50, 100 ppm – of essential oil on two-day-old adult worms, until they reached their last stage. They performed the lifespan experiments using tissue culture plates maintained at a constant temperature of 20°C. The researchers counted live and dead worms hourly and found that the lifespan of the worms at a concentration of 10 ppm increased by nearly 20 per cent!

The team then treated adult worms with juniper oil for three days and exposed them to a temperature of 37°C. They scored viability/hour to evaluate thermo-tolerance. The scientists recorded the survival responses of the worms by touch-and-provoke method using a platinum loop. They found that the worms survived thirty per cent more than untreated worms when exposed to heat stress.

The team then examined *in vivo* antioxidant activities. They found that worms treated with juniper oil had 40 per cent more antioxidant enzymes than controls.

India has more than a hundred million people aged above 60 years. So the essential oil of juniper berry promises a market for developing more functional foods, nutraceuticals and anti-ageing products.

However, the plant has restricted geographic distribution and grows primarily on mountain slopes, making it difficult to access. Moreover, the cones of the conifer are known for slow maturation, often taking two years. This calls for promoting its large scale cultivation and developing sustainable harvesting strategies.

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## Save Guggul

*Policies for posterity*

Guggul is an important medicinal plant used in the Indian systems of medicine. It finds use in treating a variety of disorders. Thus, there is heavy demand. The oleo-gum resin tapped from the bark is the commercial product. Commercial tapping of trees for gum affects growth. Over tapping leads to tree death. Slow-growth and associated poor seed set also endanger the species.

Guggul grows in arid and semi-arid regions, with restricted distribution. And the gum harvest is from the wild, both for export and domestic use. Its threat status has shifted from Data Deficient to Critically Endangered as per IUCN records. We need a better understanding of the global demand and supply of the species.

Geethaka99, Via Wikimedia Commons

Ravikiran Kulloli, from the Botanical Survey of India, along with researchers from Australia, the US and Germany reviewed the cultivation status and global trade of the gum. They also reviewed the impacts of oleo-resin harvest. They analysed policy decisions related to harvest and trade in the resin. The team wanted to assess the sustainability of the global trade in the oleo-resin.

The team reports that, in India, there is a decline in gum production. In Gujarat, for instance, they note a sharp decline from thirty tonnes in the sixties to 1.6 tonnes now. With reduced production on one side, and heavy demand on the other, India is forced to import the gum from Pakistan, say the authors. India then exports the processed and finished products using incorrect trade codes.

Rajasthan now has a programme called the *Guggal Bachao Abhiyan* that includes conservation, education and planting. There is also increased emphasis on developing scientific and sustainable tapping methods. Local resource management institutions are increasingly involved in improving public awareness.

Despite all possible efforts to conserve the plant, India is no longer self-sufficient in guggul resin production, and the international market for the resin is on the rise. Hence, a policy has to be evolved such that over-exploitation of natural sources reduces, feel the researchers.

'The CITES Appendix II listing could be a good policy option to safeguard the remaining stocks of guggul', says Kulloli.

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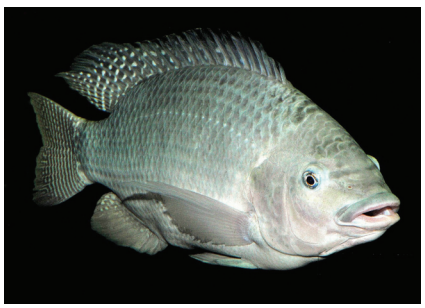
## Probiotic Protects from Pathogens

*Fish farmers find benefits*

Aquaculture has nutritional and economic importance. Regrettably, bacterial infections affect aquaculture productivity. Though antibiotics may alleviate the problem, bacteria soon develop multidrug resistance. This calls for other means of pathogen control for aquatic lives.

Recently, scientists from the Mahatma Gandhi University, Kottayam, Kerala, found a solution to the problem. They identified a probiotic bacterium – *Bacillus coagulans* – that can overcome the pathogenicity in Tilapia – *Oreochromis niloticus* – an economically important fish species cultivated worldwide.

'Though we know that probiotics affect the species, there are no reports of their ability to protect fish against pathogens', says Sebastian Midhun, Mahatma Gandhi University.



Germano Roberto Schüür, via Wikimedia Commons

The team first assessed the antimicrobial properties of *B. coagulans* against six pathogens. Cell-free bacterial extracts, they found, inhibit the growth of all six pathogens. This meant that the probiotic bacteria were releasing factors that affect the growth of fish pathogens.

The researchers, then, tested the ability of this probiotic bacterium to form biofilms – an essential criterion of a probiotic to colonise fish intestines, to fight pathogens. Using a scanning electron microscope, they could see that the probiotic bacteria do indeed form biofilms.

They also detected some enzymes, secreted by the bacteria, which can digest carbohydrates, proteins and lipids.

'The probiotic bacteria, capable of producing these enzymes, not only protect fish from pathogens but also facilitate feed digestion and aid faster growth and development of fish', says Mathew Jyothis, Mahatma Gandhi University.

The scientists proceeded to determine the ability of this bacterium to colonise fish organs. They grew the bacteria in mucus extracts of intestine and skin. The bacterium grew better on intestinal mucus than on skin. What is more, this bacterium exhibited tolerance against high concentrations of bile juice from fish. This indicated the ability of *B. coagulans* to adapt to the fish intestine.

However, injecting *B. coagulans* into *O. niloticus* can cause pathogenicity. To look into the matter, the scientists injected the bacterium and observed the fish for a month. They did not note disease symptoms.

The bacterium can be multiplied easily for field applications since it can form spores. This makes it cost effective to counter infections in aquaculture. The researchers hope that Tilapia farmers will benefit from their study.

*Bacillus coagulans* has been used as probiotic for humans to ward off diarrhoea, bloating and gas formation. So introducing the bacteria into an aquaculture environment will not be harmful to humans and may even be beneficial.

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## Karanj Oil Fuel

*Soy-lecithin bio additive*

Image: V. Anoop Kumar

Vegetable oils can be directly used as biofuel – an attractive alternative to diesel in these times of rising fuel prices. But how does long-term use affect engines?

Recently, scientists from the IIT Bombay and Cummins Technologies India Private Ltd, Pune explored the effect of the long term use of karanj oil as fuel in a diesel genset. They also investigated the effects of applying soy-lecithin, a by-product from soy oil production, often used as additive to improve performance.

The scientists filtered the oil and added 0.2% of soy-lecithin. After stirring the mixture, they poured it into the fuel tank and maintained the tank at a temperature of 65°C.

The researchers operated the genset at a constant speed and injection pressure for 130 hours. They measured exhaust emissions, smoke opacity, carbon deposits and brake thermal efficiency.

They conducted the tests without additive, and with continuous dosing of the additive. The researchers found that engine performance reduces with and without additive. They found more smoke opacity during both conditions.

However, when they dosed the fuel with the additive periodically, engine performance improved. They hypothesised that dosing for short periods allows the cleaning up of deposits formed by the oil while continuous dosage leads to the formation of deposits through charring. When they checked, they found that periodic addition not only reduced smoke opacity but also improved brake thermal efficiency.

So they recommend dosing the additive periodically. 'Run the engine for 7 to 8 hours with only karanj oil. For the next two hours, run it along with the additive for better performance', says Shah, IIT Bombay.

The advice could be exploited in remote and rural areas for irrigation, decentralised power production, water supply and other purposes.

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## Quest for Rechargeable Cells

*Lithium sulphur combination*

Lithium–sulphur composites have transformed batteries over the past decade. Lithium–sulphur batteries are economical, lighter and offer high energy density. Such features are in high demand.

However, lithium–sulphur batteries are unstable. Polysulphides that form during every battery cycle dissolve the cathode: electrodes degrade fast. This affects battery efficiency over time, preventing large-scale commercial exploitation of the technology.

Last fortnight, researchers from the IIT Kharagpur reported developing a prototype of a lithium–sulphur based composite cathode with improved electrochemical activity. They impregnated titanium dioxide, the base of the cathode, with sulphur. Titanium dioxide is polar. So it adsorbs sulphur better, boosting discharge capacity. But it also weakens electrical conductivity.

To keep the electrical pathway between sulphur and titanium oxide strong, the researchers used reduced graphene oxide. And to reduce the loss of sulphur during discharge cycles, they coated the sulphur on the cathode with polyaniline.

The researchers say that even after twenty cycles, the discharge capacity reduced only by less than three per cent before stabilising. In available lithium–sulphur batteries, efficiency reduces drastically within the first few cycles before stabilising.

The cathode composite not only offers better electrical conductivity but

is flexible. So, beyond improving lithium–sulphur batteries, the research may lead to new applications.

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### Sensible Smartphone

*Detecting urea in saliva*

Urea is a major product of nitrogen metabolism in humans. It is eliminated from the body mainly by the kidneys through urine. Urea levels in body fluids, such as blood and saliva, rise drastically under certain kidney dysfunctions. Heart failure, hypovolemic shock, gastrointestinal bleeding, and severe infections can also lead to a rise. Thus, urea in blood and saliva provides key information on renal function and helps diagnose various disorders.

Most methods for estimating urea in body fluids are based on colorimetry. These methods are time consuming and involve painful blood extraction. Collecting saliva is non-invasive. And research has correlated salivary and blood urea levels.

Recently, scientists from the IIT-D and the AIIMS, New Delhi successfully developed a smartphone-based optical biosensor to detect urea in saliva. To fabricate the sensor, they directly immobilised the urease enzyme with a pH indicator on a filter paper-based strip. As response to the urea in saliva, the paper strip changes colour. The red, green and blue levels help measure urea concentration.

The scientists used the slope method, sensor response change per unit time, instead of the differential method, the difference in sensor response between two-time intervals, to increase sensitivity and eliminate interference by variations in ambient light.

The team clinically validated spiked saliva samples and samples from healthy volunteers.

Image: Victorgrigas, via Wikimedia Commons

The smartphone application with paper strip kit can even be operated by non-professionals with limited training. This saves time and cost spent on bulky spectroscopic procedures. The report can revolutionise medical screening of large populations. And, such mass screening of diseases would boost national health.

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### Vocals and Music

*Separating automatically*

Every day, large amounts of music are uploaded into digital clouds. Often, soundtracks lack essential metadata, such as track, singer or composer name. This makes it difficult to search for and retrieve the music. So researchers started investigating methods to automatically extract metadata.

Before learning to recognise singer from voice, the machine needs to know where the vocal begins and which parts have only instrumental music. Attempts to achieve this have not succeeded completely because instrumental music continues as background when the voice begins and, thus, intervenes in the process of separation – even in typical stereo track recordings.

Srinivasa Murthy and Shashidhar Koolagudi from the National Institute of Technology Karnataka, Surathkal, have now come up with a method to overcome the problem. They note that singers intentionally change pitch to provide music with a melody component. Moreover, resonances in human voice production vary as frequencies increase, more than those of sounds made by instruments. By analysing voice and instrumental formant features, the researchers identified five such features that distinguish human voices from sounds produced by instruments.

To train learning machines, the team then took short clips of sound tracks with only instrumental music as well as clips with vocals. They tried various existing automatic classifiers including support vector machines, neurofuzzy classifiers, as well as random forest and artificial neural networks. Artificial neural networks, they found, work well to extract features that distinguish vocals from instrumental segments.

An artificial neural network has an input, a hidden and an output layer. By rigorous experimentation, the team determined that neurons in the hidden layer should be 1.85 times that of the input layer for optimal results.

The features extracted by the learning machine were fed into a genetic algorithm. The algorithm selected 10 'fittest' features.

The team then tested the ability of these features to distinguish vocals and instrumental segments in the standard dataset containing a thousand music clips. Taking a frame length of twenty-five milliseconds and an overlap of ten milliseconds, the system had to deal with a 93-dimensional feature vector for each frame. The large dimensions of the feature vectors increase the need for computing power.

This can indeed be decreased. 'Just three features were adequate to distinguish vocal and non-vocal sections in the standard dataset', says Srinivasa Murthy.

'But Indian music is more complex. To distinguish vocal and non-vocal regions in Bollywood and Tollywood songs, we need at least seven features. That means a feature vector of 74 dimensions', adds Shashidhar Koolagudi.

By carefully selecting features that showed maximum correlations with vocal or non-vocal segments, the researchers further cut down the feature vector to 43. But that gave an accuracy of only about 84%.

Then they tried the moving window approach. An 11-point moving window reduced the feature vector dimension to 32 – optimal to operate even with music videos calling for higher processing powers. Accuracy improved to more than 95%.

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*Reports by:* **Sileesh Mullasseri, Rekha R Warrier, Siva Shakti, Sanghamitra Deobhanj, V. Anoop Kumar, P. K. Udham, S. Balaji, Gita Madhu** and **Pavithra Naik**

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scienceandmediaworkshops@gmail.com