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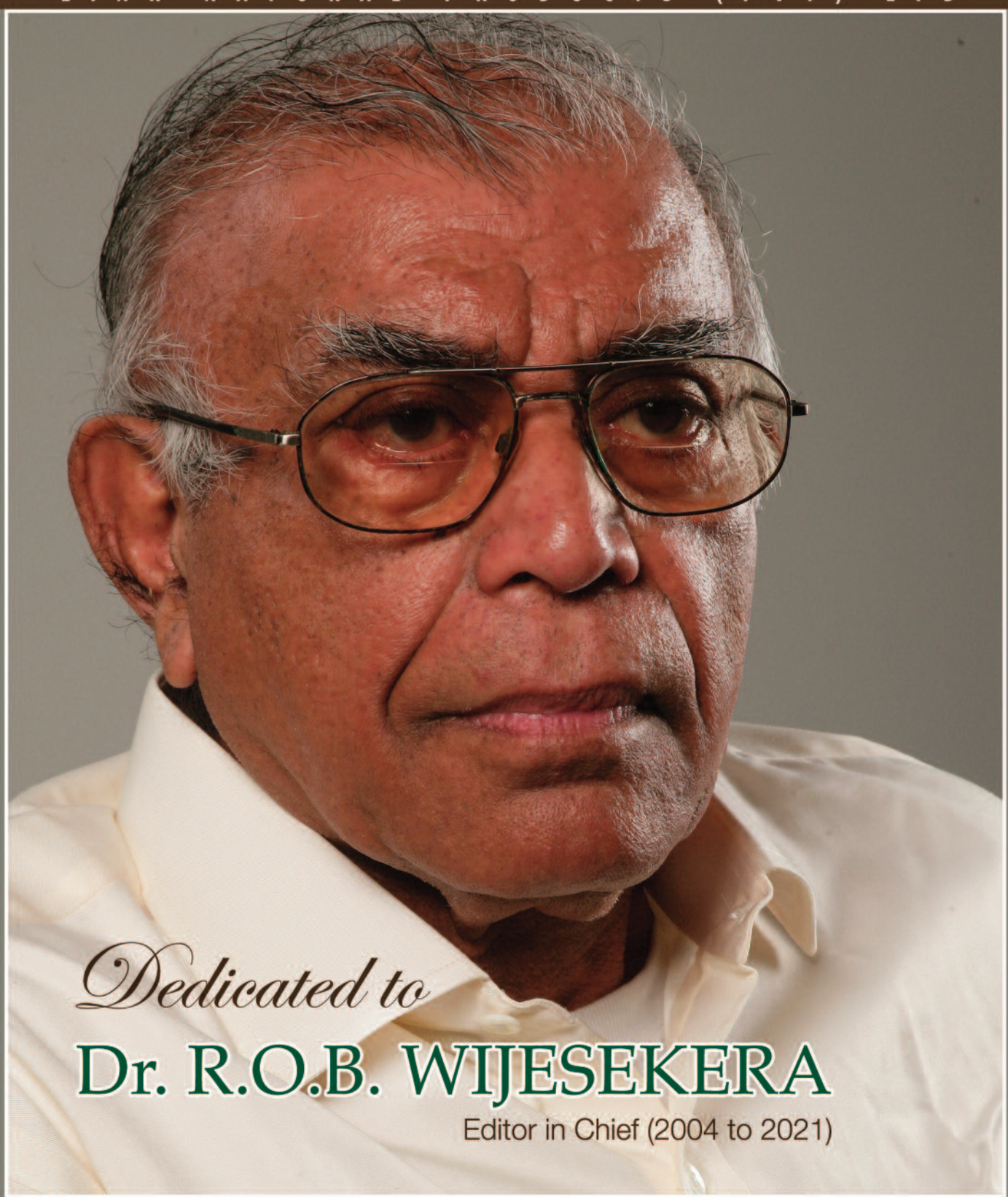

Dedicated to

Dr. R.O.B. WIJESEKERA

Editor in Chief (2004 to 2021)

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IN THE PAGES THAT FOLLOW.....

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EDITORIAL

The inaugural issue of the Link Natural Products Digest (LNP Digest) was in October 2004, with Dr. R.O.B. Wijesekera, as the editor-in -chief. It was very much a creation of Dr. Wijesekera's where he determined its structure, format and scope of content. Its readability and quality ensured the LNP Digest a regular readership, and also elevated the profile of LNP as a science driven organization which manufactured herbal products, drawing inspiration from traditional knowledge.

In addition to his editorial functions, Dr. Wijesekera also contributed entertaining and informative articles on various aspects of medicinal plants and essential oils to the LNP Digest. He also wrote a regular series on "Celebrated Researchers" starting with Finn Sandberg in issue 2, volume 2, (2006) The last article in this series (No 22) was in issue 1, volume 16 (2020) (Torbjörn Norin). These articles were based not only on his knowledge of researcher's scientific contributions but also on his personal knowledge of the researcher. Sadly, this insightful series now comes to an end.

The current editorial board appointed in 2022 after Dr. Wijesekera's demise, has introduced a new feature titled "Complementary Perspectives", which will present perspectives from Modern Science and Ayurveda, on topics related to medicine. The first of a series of three on Arthritis, appears in the current issue. It is hoped that young medical graduates will find inspiration for trans-disciplinary and inter-disciplinary research from these articles.

The editorial board is honoured to dedicate this issue of the LNP Digest to its founder editor-in-chief, Dr. R.O.B. Wijesekera

Ajit Abeysekera

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MESSAGE FROM THE CHAIRMAN, LINK NATURAL PRODUCTS

Dr. R.O.B. Wijesekera made an immense contribution to the essential oils and natural products industry in Sri Lanka. I am indebted to him for the support and help he provided to Link Natural Products in its fledgling stages, without which the company would not be what it is today. With his extensive knowledge of the herbal products industry, he was a continuous source of novel ideas for development. Over the years, he became a person to whom I could always turn to, for mature judgement on both personal and professional matters.

He was the initiator of the Link Digest and was its editor since its inception. The high quality of his contributions to the journal and the esteem with which Dr. Wijesekera was held in the scientific community, helped in raising the profile of Link Natural Products.

I am pleased that this issue of the Link Digest is dedicated to the memory of Dr. R.O.B. Wijesekera, a true gentleman of science and a friend of the Company.

Dr. Devapriya Nugawela

Dr. R.O.B. WIJESEKERA: A SCIENTIST WITH HUMANE QUALITIES

Dilmani Warnasuriya

Dr. R.O.B. Wijesekera, the doyen of Natural Products Chemistry, and a much loved figure among all those he worked with passed away on the 13th of July 2021 after a brief illness. He was 92 years at the time of his demise, and knowing his abiding love of cricket, he would have said with a satisfied smile, "I have batted a good innings".

Dr.Wijesekera's illustrious career as a natural products chemist and research manager is well known among the scientific community. But not many know about his endearing qualities as a human being. I will endeavor to give justice to both these aspects.

Dr. R.O.B. Wijesekera started his research career at the MRI in 1952 where he worked on indigenous medicinal plants. He proceeded for his PhD to the University of Sheffield in 1955 and returned in 1959. He joined the CISIR in 1966 and built up a vibrant and active Natural Products Section producing research papers and patents at a prolific rate, and gaining much fame in the field of spices and essential oils. His immense contribution towards human resources development was the concept of a two-part Ph.D. system where several of his protégés were sent to spend brief periods to European laboratories by arrangement with his colleagues at foreign universities. Their work was then used for presentation of doctoral theses at local Universities. He was thus responsible for the conferment of PhD s on several of his staff, for which their gratitude is palpably displayed by their devotion to him. Although scattered far and wide across the world they never fail to pay homage as it were to their beloved guru on their regular visits to their homeland.

For the performance of research in this sector, and his leadership he was awarded the Guinness Award for Scientific Achievement in 1976 administered by the Commonwealth Science Council. The citation starts off with "The Sri Lankan Scientist to receive the award is Dr. R.O.B. Wijesekera of the CISIR. Dr Wijesekera has for the past ten years built up a unit to study and develop the extraction of essential oils from plants notably cardamom, citronella, and cinnamon,….."

Dr. Wijesekera's international career took off after he receivd the Guinness Award, when he served as a consultant to UNIDO-ESCAP on the essential oil industry. He went thereafter to Guyana as Special Consultant to the Government and in 1978 he was selected by the WHO as Task Force Manager of their International Research Programme on Plants for Fertility. Having proven himself as a able research manager, he was recruited by UNIDO in 1980 as a Special Technical Advisor to the Chemical Industries Branch and was responsible for the Industrial Utilisation of Medicinal and Aromatic Plants programme in several countries. He was instrumental in initiating major UNIDO projects in the areas of essential oils and medicinal plants in several countries, mainly in Asia and Africa. His extensive travels into the exotic and little known areas in the world is vividly described in his autobiographical treatise titled' Clouds are not spheres nor mountains cones' which was published in 2011.

After his return to Sri Lanka, Dr. Wijesekera came back to CISIR (then ITI) as the Chairman, in 1995, before taking over as the inaugural Chairman of the National Science & Technology Commission (NASTEC), when the S&T Development Act came into force in 1998. Dr. Wijesekera was awarded an honorary D.Sc from the University of Sheffield, a D.Sc. Honoris Causa by the University of Peradeniya, was a Fellow of the Royal Society of Chemistry and a Chartered Chemist of the U.K., and Fellow of the National Academy of Sciences (Sri Lanka). He was a past President of both the Institute of Chemistry and the Sri Lanka Association for the Advancement of Science, and was also the recipient of the Presidential Vidya Jyothi Award. He was much sought after as a Consultant in both the private and public sector. Almost till the time of his demise he served as Director/Consultant at Link Natural Products.

It is at Link Natural where I worked as the Editor of the Link Natural Digest with him as the Editor in Chief that I really got to know Dr. Wijesekera on a personal basis and I learnt so much from him. However much he aged, his love for reading scientific material never abated, and his innate ability to just skim through an article and identify areas of research potential never failed to amaze me. He was a scientist with an incredible vision and he had an uncanny insight into what subjects would draw the interests of the readers when compiling the Digest. During his tenure as Editor Chief of the Link Natural Digest, the accolades he received from eminent scientist all over in the world are indeed gratifying.

Another unique characteristic he had was his phenomenal memory. He could recall events of his childhood as if they happened just the other day and even quote verbatim conversations that took place. At first I was skeptical and thought it was just a figment of his imagination, but when it was repeated (on several occasions actually) the content was the same. The most endearing quality about him was his genuine concern for the welfare of his staff, professional and domestic, going to extraordinary lengths to assist them in any way he could. As for me, he leaves an irreplaceable void in my working life as a mentor and sounding board. He influenced my thinking patterns immeasurably. As for his personal life, he was very much a family man and was an amazing husband, father and grandfather. He was so proud of their achievements and would relate so many stories about them that I felt I knew them personally. So much so that even before I met them, I'm sure I would have been able to identify each one of them if I saw them for the first time.

Finally, I think he would also want it mentioned that he was an FRCS , not a medical one but a Former Royal College student who distinguished

himself both as a scholar and in sports having represented his school in cricket and rugger.

Dr. Wijesekera led a good life, ever willing to lend a helping hand to those in need, and I know he will reach a place where his actions will be richly rewarded. May he attain the supreme bliss of Nibbana.

Dr. R.O.B. Wijesekera
Ph.D. DSc, FRIC, FI Chem C, CChem.
IN APPRECIATION OF AN OUTSTANDING MENTOR AND A GOOD FRIEND

A.Lakshman Jayewardene, Ph.D.

I first met ROB more than fifty years ago, soon after his return from USA after a post-doc year at the University of California, Davis campus in 1969. When I applied for a research officer post in Natural Products at the Ceylon Institute of Scientific and Industrial Research in Colombo, Prof. Percy Wannigama in the chemistry department at the Peradeniya campus told me, his class-mate ROB Wijesekera was the head of the Natural Products Section (NPS). When I joined the NPS ROB was on his way to Davis, California and one year was to pass before I met him. Dru Fonseka from the University of London had joined the Fats and oils section the previous year and the two of us worked diligently to get an old Unicam-Pye gas-chromatography instrument into working order. When ROB returned he was able to garner sufficient dollars to purchase a set of the latest Varian gas-chromatography (GLC) instruments and an Infra-red Spectrometer. ROB drafted a program for the systematic study of the chemistry and technology of the local growing aromatic plants and spice varieties. We were a small group of four graduate research officers and a few laboratory technicians and a secretary-clerk. Within a few months we had the new instruments in working order and began analyzing the volatile constituents of locally produced aromatic essential oils. The field engineer from Varian company came a few months later and just had to sign the papers to formalize the warranty. During the post-doc year at Davis ROB had used the very same GLC equipment to analyze the constituents of essential oils of the valuable spice cardamom growing in Sri Lanka, India and Guatemala. At the NPS we then began a systematic examination of the volatile constituents of our own Ceylon cinnamon bark and leaf. Another of our efforts was the commercially produced essential oil from citronella grass that had a very low market value due to the stigma of adulteration that was attached to this product. This story was legend in the essential oil markets around the world. The sole basis of this slander was the quality test on which citronella is judged, this is the infamous Schimmels test which is a solubility test in ethyl alcohol of various strengths. The citronella oils from many other countries all passed the Schimmels test using 80% ethanol in water, whereas even freshly distilled citronella oil from plantations in the south of Sri Lanka invariably failed this test. One text book on essential oils quote instances where the oil sellers were asked if they were selling citronella oil or kerosine. ROB, Dru Fonseka and the author worked on this problem and very quickly found that Ceylon citronella oil contains a fairly large percentage of terpene hydrocarbons and much less oxygenated terpene compounds than the oils from other countries. It is well known that terpene hydrocarbons do not dissolve in aqueous ethanol consequently Ceylon citronella oil will not pass the Schimmels. We were also able to show that there was no so-called adulteration with kerosine, based on a simple test using a gas chromatograph. After these results were published in the Phytochemistry journal it was gratifying to note the improved market value of our local oil of Citronella. The Tropical Products Institute in London organized an international

conference on spices in 1972 and ROB and the author were able to attend this meeting to present our research on local spices and essential oils to this forum. These were difficult days for a small developing country with dire shortage of foreign currency so much so that each of us was allowed only a measly five US dollars for the whole visit to the UK. The British Council and the Commonwealth Foundation gave generous funds to support us. I remember very clearly that after ROB presented our paper, a member in the audience commented that the market price of spices from Ceylon were quite high. ROB's response was "I can only quote what a merchant from China said, "Good Things Not Cheap and Cheap Things No Good," the organizing secretary was so thrilled with this repartee that local newspapers in London mentioned this quote during reporting on summary of the proceedings of the conference.

ROB had contacts in many places high and low in the government and in scientific circles around the world. When the prevailing economic problems caused many training programs for young scientist to be curtailed, he told me that his research section was cut off from the available scholarships for foreign training. However within a short time he was able to organize a one year training program with the help of his friend Finn Sandburg from the University of Uppsala, Sweden under the auspices of the Swedish International Development Agency (SIDA). This International Seminar in Chemistry as it was called was very successful and valuable. It facilitated the training of five other young research officers who were invited to participate in various pharmacognosy programs. Two of them were able to apply the results of this training to part fulfill the requirements of doctoral programs jointly with local universities in Sri Lanka. I was able to use the research results from my work on Spices and Essential oils under the mentorship of ROB, to submit a dissertation for a doctorate. ROB was also concerned about the technology of the essential oil industry which was of the early twentieth century vintage. A survey of the equipment used in rural Sri Lanka for distillation of oils of Citronella, Cinnamon bark and Cinnamon leaf oils indicated no modern stills or boilers for steam generation etc. were being used at this time. Many of the still bodies were made of timber and the steam for the distillations were generated in steel drums, fired with fire-wood and dried spent leaves or spent grass from earlier citronella distillations. He instituted a program for the development of new technologies for processing local aromatic plants and spices. Cisirill Manakoka still (ROB's name for this equipment) was specially designed for use in dry-zone rural areas where water for cooling the condensers was scares. His team also designed a distillation unit for fine spices called SPICA which was originally heated with electric heating elements, unfortunately the prevailing economic conditions obligated that the locally manufactured heating elements had to be installed and there was catastrophic failure of these poor quality elements within a very short time. As a remedy LP gas heated SPICA stills were manufactured in small numbers. This research and development work was reported at the International Congress of Essential oils in 1974 in San Francisco. In 1975 ROB led the Sri Lankan scientific team opposite Prof. Carl Djerassi and his team during the US/Sri Lanka Workshop on Natural Products held in Colombo.

The innovative and productive research work performed by Dr. R.O.B. Wijesekera was recognized internationally by the presentation of the Guinness Award for Scientific Achievement by the Commonwealth Science Council in 1976. He was the first Sri Lankan to win this award for research performed solely in the country. In the same year he was chosen as a specialist consultant expert by UNIDO-ESCAP. What did this pioneering and hard work get us from the

trade and government of Ceylon? Absolutely nothing, no words of appreciation. There is a saying at the American Chemical Society to the question, 'why do chemists practice their profession so diligently"? the answer is because they pay us to do what we love to do'. ROB and his team did not stop at this point, but continued to develop new technology and generate new knowledge about aromatic plants and spices, after ten years of selfless service it needed a foreign organization to recognize these efforts. As mentioned above ROB was awarded the coveted Guinness Award for sustained R&D efforts in a developing country. ROB left Ceylon soon after, but he did not give up on the people of Sri Lanka. Many opportunities were found for young scientists to be funded to start projects or opportunities to work as consultants in other developing countries, while he worked for the different branches of the United Nations Organization. Finally ROB wrote me a note indicating his final retirement from full time work and that he and Marina will be returning to Sri Lanka. Characteristically it was not to idle away the well earned rest, but to work in a consultant position with over fifty years R&D experience. Link Natural Products (Pvt) Ltd, gladly took him on as a member of the board of management. On my retirement from the University of California, San Francisco Campus I too was invited to join the company. I am happy to mention that over 25 years ago the Chairman and MD of Link Natural Products asked me for help in setting up the small essential oil producing facility. It is with great pleasure that I rejoined that small venture which has now grown to be an industry leader in spices and essential oils and scientifically formulated herbal health products which have international recognition. I can say with confidence that ROB's saying is very much in force at this industry that, "good things are not cheap and cheap things no good". Link Natural Products has a well equipped quality control and R&D laboratory and a team of qualified scientists working all the time assuring the quality of raw materials, intermediate and final products manufactured at this facility. The raw materials are the key to running a profitable and well regarded industry and I personally know the great effort needed to maintain the high standards for which this company is recognized. The list below is the ROB's R&D team and many others who helped us in many ways. I would also like to pay a personal tribute to Prof. Tuley de Silva who was in the faculty of Applied Science at the University of Sri Jayewardenapura whose vision of Industry/University joint post-grad research program enabled me to register for my doctoral degree and I was the first in the chemistry program.

The R.O.B. Wijesekera R&D Team 1969 – 1977: Late Upali Senanayake, late Drupadha Fonseka, Lakshman Jayewardene, Ph.D. Lakshmi Rajapakse-Arambewela, Ph.D. Kanthi Fonseka-Hettiarachchi, Ph.D. late Roshantha Chandraratne, Anura Senaratne, late Donald Wijekoon, V. U. Ratnayaka, Azeez M. Mubarak, late K. Ratnasingham, Philomena Ambrose, Lakdas Fernando, Nissanka de Silva, Kamalita Fernando, Ranjit Dayananda, late Miss C.L.M Nethsingha, Arthur Bamunuarchchi , Shiranee Samaranayake, PCM Fernando and Mr. Monis, our lab-attendent. Many of the research officers and graduate students were able to complete doctoral programs later in laboratories in the developed world. One of ROB's last activities was taking on the task of chief editorship of the Link NP Digest, which he continued to with able assistance of Dilmani Warnasuriya until the very last weeks of his long and successful career.

During his Chairmanship of the ITI (former CISIR) he associated with Mr. Dayananda and Dr.Upali Senanayake to write chapters for the renowned book published on Ceylon Cinnamon "Cinnamon and Cassia - Genus of Cinnamomum" edited by Prof P N Ravindran by CRC press.

Accolades from far and wide have been written about him and his part autobiography 'Clouds Are Not Spheres, Nor Mountains Cones' is subtitled, A scientist's personal kaleidoscopic story of professional & family life in an international milieu.

One of his later collaborators says this “What a gentleman Dr ROB was, he never ever tried to find the wrongs of others, helped all who wanted to work hard and deliver for the country, always without bias, not considering how fluent or not in English or what level of society they came from."

Farewell ROB, we will all remember you for the great work you did for us and the country and your qualities of leadership, and as a teacher. A man who deserves the highest respect from all who knew him.

May your soul rest in peace

PRELIMINARY STUDIES ON THE STABILITY OF A MOISTURIZING CREAM CONTAINING ORANGE JASMINE FLORAL ABSOLUTE (*Murraya paniculata* (L.) JACK)

T.M.S.G. Tennakoon, Director, R&D/QA, Link Natural Products (Pvt.) Ltd.

Abstract

The floral absolute obtained by fractionation of the n-hexane extract of orange Jasmine (*Murraya Paniculata* (L.) Jack) with cold ethanol, was incorporated in a moisturizing cream.

Preliminary organoleptic and physico-chemical tests indicate that the formulation is stable.

1. Introduction

Orange Jasmine (*Murraya paniculata* (L.) Jack. 'Etteriya' in Sinhala) is a shrub or small tree distributed in India, Sri Lanka, Burma, Indo-china, Malaysia and Australia. In Sri Lanka, the plant is found in the low country, wet and (partly) dry zones, up to 1000 m. It is also grown as a house plant and as a landscaping plant. The major compounds in the essential oil of leaves from plants growing in India, Nepal and Cuba have been reported as β-caryophyllene, β−cyclocitral, methyl salicylate, trans−nerolidol, methyl palmitate, isospathulenol, trans-verbenol, trans-verbenol and germacrene-B. The oil has nematicidal activity, antioxidant properties and antimicrobial activity (Dosoky *et al*., 2015, Arya *et al*., 2017). The fruit essential oil mainly contains, (E) – nerolidol (25.7 %), manool (18.7 %), benzyl benzoate (8.1 %) and phenyl ethyl benzoate (8.0 %) (Olawore *et al*., 2005). The flower oil is dominated by manool, phenyl ethyl benzoate and (E)−nerolidol. The major components in the concrete and absolute are manool, phenyl ethyl benzoate, (E) – nerolidol and benzyl benzoate (Rout *et al*., 2002, Rout *et al*., 2010).

Despite the availability of synthetic fragrances, there is a continuous demand for natural fragrances in the cosmetics industry, particularly as an ingredient for high valued products. Floral absolutes such as jasmine and rose are in high demand. In house sensory evaluation of the floral absolute of orange jasmine suggested that it could be a viable substitute for jasmine absolute. The stability of a fragrance material in a cosmetic formulation is an important requirement for the quality of the formulation.

2. Materials and methods

Preparation of floral concretes and absolutes

Fresh orange jasmine flowers (2.2 kg) were collected between 6.00 pm and 10.00 pm from Dambukanda herbal garden at Link Natural Products (Pvt) Ltd, weighed and placed in a 50 L

extraction vessel and extracted four times with n-hexane (10 L) at room temperature. The contact time was 45 minutes for the first extraction and 35 minutes for each of the following extractions. The extracts were filtered through Whatman No. 1 filter paper. The solvent was evaporated from the combined filtrates under vacuum at 40 ^{0}C to obtain 96.8 g of floral concrete.

The concrete (90 g) of orange jasmine obtained above was dispersed in 96 % (v/v) ethanol by agitation and then kept at -8 ^{0}C for 24 hr. The ethanol soluble portion of the concrete was separated by filtration through Whatman No. 01 filter paper. The extraction of the concrete with alcohol was repeated two more times. The three filtrates were combined and the solvent was evaporated under vacuum at 40 ^{0}C to obtain the floral absolute (22.20 g) as a thick pourable liquid.

Preparation and stability testing of a topical moisturizing cream containing the floral absolute of Orange Jasmine.

All the excipients used were cosmetic grade chemicals from Croda, Dow chemicals, and Lubrizol. A RQT-127/D medium duty high speed emulsifier with 1/8 motor, digital speed Indicator and transformer speed regulator was used.

A 10 kg batch of Topical cream of O/W emulsion was prepared with the floral absolute of Orange Jasmine having the composition given in the table1.

Table 1. Composition of topical cream

Ingredient	% w/w
Phase A	
Steareth-2 (HLB=2)	3
Steareth-21 (HLB=15.5)	2
Cetearyl alcohol	1
PPG-15 Stearyl Ether	4
Stearic acid	1.5
Phenoxyethanol and ethylhexaglycerin	1
Phase B	
Water	82.75
Carbomer	0.25
Glycerin	4.00
Phase C	
Murrya paniculata floral absolute	0.50

The ingredients of phase A and phase B were first mixed separately (40 rpm) and heated to 70 °C to obtain two clear solutions. Phase A was gradually added to phase B during 5 mins while homogenizing at 400 rpm. After completing emulsification, the speed was brought down to 40 rpm and continued until the emulsion cooled to 40 °C . The floral absolute was then added and mixed at 40 rpm for 5 min and the pH value was adjusted by adding 10 % (w/w) citric acid solution to 5.5 ± 0.5 .The emulsion was kept for 24 h at ambient temperature for stabilization.

Preliminary stability testing:

The preliminary stability tests were carried out according to the ANVISA Stability Guidelines, 2004. Topical cream was subject to a freeze thaw test. The heating and cooling cycle was carried out using a cooling incubator and the temperature of the test sample was changed between -5 ± 2 °C and 45 ± 2 °C every 24 hours during a period of 12 days. Organoleptic parameters, physicochemical parameters and the HPLC chromatogram were evaluated at the commencement of the test and after the 6th day and the 12th day. The tests were carried out in triplicate. The test samples were compared to the standard samples. The standard samples were samples drawn from the bulk topical cream prepared for testing, but kept at room temperature, protected from direct sunlight and humidity.

Organoleptic Parameters

Appearance, colour and odour were evaluated as organoleptic parameters for the stability of the topical creams. The parameters were graded as follows.

Appearance :

Normal - No change observed.
Slightly separated – Slight precipitation or slight turbidity observed.
Separated - Definite precipitation or turbidity observed.

Colour and Odour:

Normal – No change observed
Slightly modified – Slight change observed
Modified – Definite change observed
Intensively modified – Marked change observed

Physicochemical parameters
pH value

One gram of the topical cream was mixed thoroughly with 9.0 mL of distilled water and the pH value was measured using a pH meter. Each measurement was carried out in triplicate.

Viscosity

The topical cream (30 g) was placed in a glass beaker and the viscosity was measured by using a Brookfield viscometer. The spindle used was number 63 and rpm was 0.1. Each measurement was carried out in triplicate.

Phase separation

The topical cream (5 g) was placed in a centrifuge tube and centrifuged at 3000 rpm for 30 min at 28 ± 1 °C. The tube was examined carefully to observe whether there was a separation of phases. Each test was carried out in triplicate.

HPLC analysis

The topical cream (1.0 g) was placed in a 30 ml centrifuge tube and 10 mL of n-hexane was added. A homogeneous mixture was obtained by thorough mixing using a Vortex mixer for 10 s and keeping in an ultrasonic bath for 3 min at 50 °C. The homogenous mixture was kept -78 °C (dry ice-acetone bath) for 2 min and centrifuged at 6000 rpm for 5 min. A volume of 7.5 mL of the supernant of n-hexane was withdrawn and the process was repeated thrice adding 5 mL of n-hexane and withdrawing 5 mL of n-hexane each time. The hexane extracts were combined and filtered through a C18 cartridge filter, followed by a 0.45 micron nylon filter. HPLC analysis was carried out by injecting 25 µL from the combined extract using the following (table 2) gradient at a flow rate of 1.0 mL/min, with monitoring at 296 nm (Desmedt *et al*., 2015, with modifications).

Table 2. HPLC gradient elution programme for analysis of topical cream

Time (min)	Phase A	Phase B (Acetonitrile) (1.0 % v/v acetic acid in water)
0	90	10
4	85	15
10	80	20
15	80	20
30	0	100
45	0	100

3. Results and Discussion

Results of the preliminary stability studies are shown in Table 3. These results indicate that the sensory, physicochemical and microbiological parameters of the topical cream are stable under the tested conditions.

Table 3 . Results of preliminary stability tests of a moisturizing cream containing Orange Jasmine floral absolute.

	Day 1		Day 6		Day 12	
Test parameter	**Control**	**Test sample**	**Control**	**Test sample**	**Control**	**Test sample**
	No change observed (Off white homogeneous cream)	No change observed (Off white homogeneous cream)	No change observed	No change observed	No change observed	No change observed
Color & Odour	No change observed (Sweet floral)	No change observed (Sweet floral)	No change observed	No change observed	No change observed	No change observed
pH at 30 ± 1°C	6.92	6.02	6.63	5.97	6.46	6.10
Centrifuge (3000 rpm for 30 mins)	No phase separation	No phase separation	No phase separation	No phase separation	No phase separation	No phase separation
Density (g mL^{-1}) at 30 ± 1°C	0.996	0.9943	0.9892	0.9910	0.9897	0.9880
Viscosity cP at 29 ± 1°C	619200	630000	587000	592800	588750	582000
Microbiology TAMC (cfu)	<10	<10	<10	<10	<10	<10
TYMC (cfu)	<10	<10	<10	<10	<10	<10

Note : TAMC = Total aerobic microbial count
TYMC = Total yeast and mold count
cfu = Colony forming unit

The HPLC profiles of the topical cream taken on Day 1 and on Day 12 (control sample and test sample) indicated that the floral absolute was stable under the test conditions. The HPLC profiles are given in figure 1

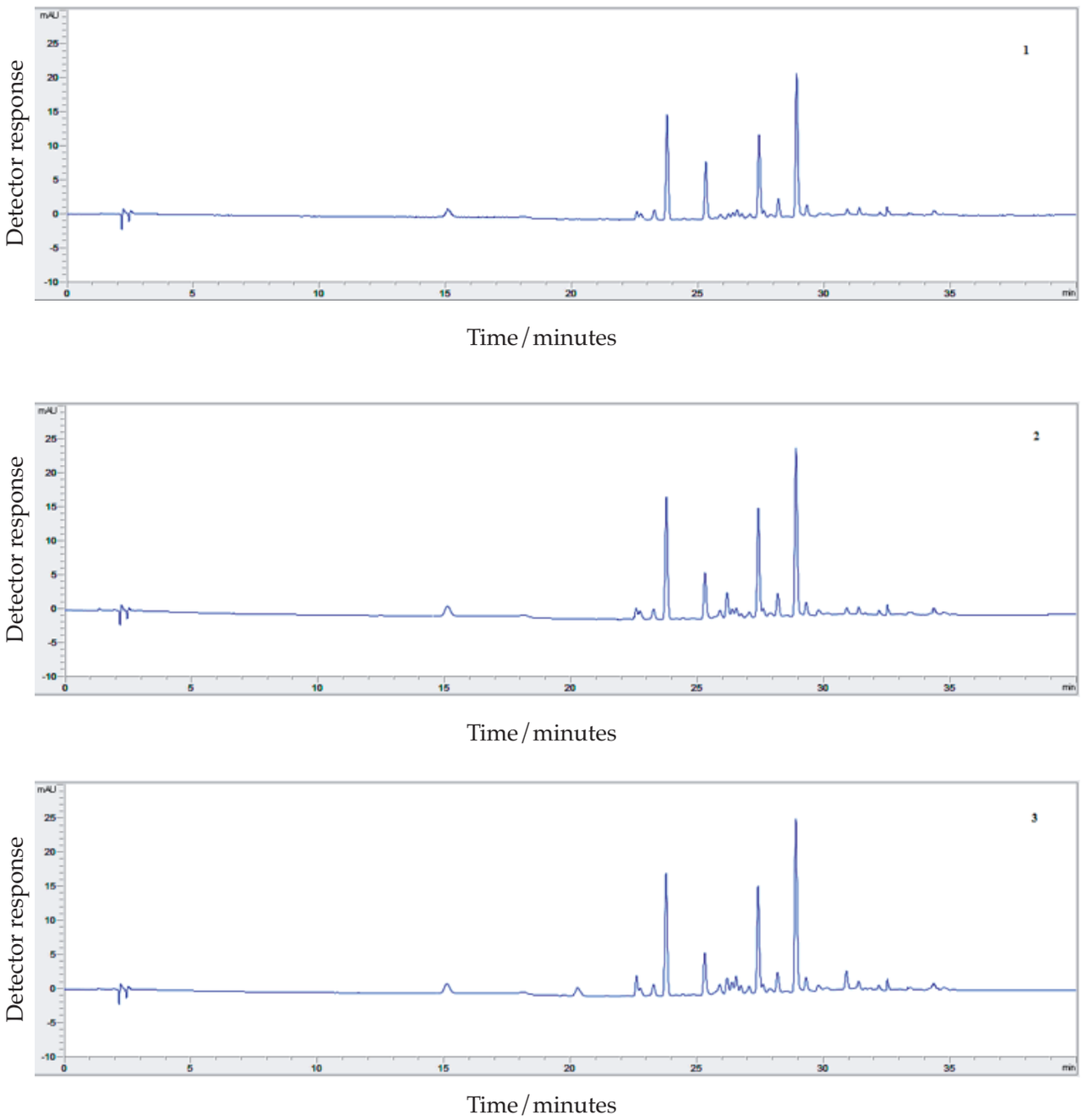

Fig 1. HPLC profiles at 296 nm of topical cream before and after preliminary stability testing

1. Day-1 control and test sample : After 24 hour at room temperature (control and test sample)

2. Day-12 : Test sample after completion of preliminary stability testing (Freeze / Thaw cycle tests)

3. Day- 12: Control samples at room temperature (30 ± 2 °C).

The overall profile of floral absolute in the topical cream did not show any remarkable differences at the end of preliminary stability testing compared to positive control at the room temperature. It was noted that relative amount of indole (Retention time (rt) = 28.87 min), the major constituent of Orange Jasmine absolute which gives its characteristic floral odor did not change significantly at the end of the preliminary stability testing.

Test parameter

Appearance

4. Conclusion

The preliminary stability tests indicate that the topical cream prepared is stable. Further long term and accelerated stability testing is necessary prior to product development.

5. References

ANVISA (2005) National health surveillance agency cosmetic products stability guide/national health surveillance,1st edition, Brasilia. http: // www.anvisa.gov.br.

Desmedt, B., Canfyn, M., Pype, M., Baudewyns, S., Hanot, V., Courselle, P., De Beer, J. O., Rogiers, V., De Paepe. K. & Deconinck, E. (2015) HS–GC–MS method for the analysis of fragrance allergens in complex cosmetic matrices. Talanta, 131, 444-451.

Dosoky, N. S., Pokharel, S. K. & Setzer, W. N. (2015) Leaf essential oil composition, antimicrobial and cytotoxic activities of *Cleistocalyx operculatus* from Hetauda, Nepal. Am. J. Essential Oils and Natural Products, 2(5), 34-37.

Arya, N., Kaur, J., Verma, A., Dhanik, J. & Vivekanand. (2017) Chemical composition of leaf essential oil of wild and domestic genotypes of *Murraya paniculata* L. J. Essential Oil Bearing Plants, 20(2), 468-473.

Rout, P. K., Barik, K. C., Jena, K. S., Sahoo, D. & Rao, Y. R. (2002) A novel process for the extraction of fragrance components from ambrette (*Hibiscus abelmoschus* L.) seeds. Organic Process Research & Development, 6, 401-404.

Rout, P. K., Rao, Y. R. & Naik, S. (2010) Liquid CO_2 extraction of *Murraya paniculata* Linn. flowers. Industrial Crops and Products, 32(3), 338-342.

DEVELOPMENT OF A HERBAL LAND LEECH REPELLENT PRODUCT

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Abstract

Land leeches or terrestrial blood sucking leeches are very common in damp forests and cause discomfort to people and livestock in those areas. The objective of this research was to study the individual efficacy of some selected plant-derived repellents in order to obtain a final product with high efficacy by combination of plant extracts. Plant extracts of *Nicotiana tabacum* and *Piper betle* and the essential oils of *Cymbopogon nardus*, *Eucalyptus globulus* and the seed oil of *Azadirachta indica* were used for the study. Laboratory tests were carried out using the treated paper rings. Many experiments were conducted to determine the minimum concentration at which the leeches do not cross the paper ring and the maximum concentration at which the leeches cross the paper ring with difficulty for each repellent. Extracts were diluted in a base of ethanol, water and isopropyl alcohol. *Nicotiana tabacum* exhibited the highest repellent activity against leeches followed by *Cymbopogon nardus.* The repellent mixture which contained the total of 10.8% plant extracts of *Nicotiana tabacum* and *Cymbopogon nardus* exhibited high efficacy and high repellency during the field tests carried out.

1. INTRODUCTION

Leech infestation and its effects in Sri Lanka

Leeches are blood-sucking worms with a segmented body that belong to subclass Hirudinea of phylum Annelida. They are well known for their sanguivorous nature which causes much annoyance and discomfort to people and other animals.[1] There are about 500 leech species known to science. The well-known are the land leeches in family Haemadipsidae which are common in South Eastern Asia, Oceania, South America, and Madagascar. These land leeches mostly attack mammals and the Ceylon Leech *Haemadipsa zeylenica* is well known among them as a serious pest to humans.[2] Sri Lanka has a high leech infestation in areas like Ratnapura, Badulla, Bandarawela, Hatton, Avissawella and Central Province where the climate is moist and humid.

Leeches mostly get attracted to the body heat of the host and especially to the areas like underarms, toe, buttock, hands and legs.[3] Once the meal is taken they leave the host.[2] Leech bites cause blood loss due to hirudin in their saliva, which is an anticoagulant, but generally the danger due to blood loss is insignificant on humans.[1][4] However there could be rare exceptions where uncontrolled leech bites can lead to coagulation disorder, consequent excessive skin haemorrhage, and anaemia requiring blood transfusion.[5]

Leeches play an important role in forest and stream ecosystem because they act as both predator and prey in many animals' life cycle. [6] Though leeches are important in the ecological balance of nature, leech infestation has become a critical problem especially for the farmers, tea pluckers, rubber estate workers and also for the people who live in those areas. It is found that use of repellents as personal protection measures is the most preferred method to treat leeches. [4]

Leech repellent products used worldwide

When travelling in leech infested areas people use various types of leech repellents to keep the leeches out of them. These repellents can be

natural, chemical or some physical methods. Some of the commonly used natural repellents are salt, vinegar[3], citronella oil, eucalyptus oil, tobacco and salt spray[7] and lime. Generally, they are applied to the skin. Since there is no specific repellent for leeches people also use insect repellents such as Mosi-Guard [3][8] to repel leeches. Also, chemicals such as deltamethrine, N,N-diethyl phenyl acetamide (DEPA), N,N-diethyl-m-toluamide (DEET)[4], dimethyl phthalate (DMP)[4], N-benzoyl piperidine (NBP),[1] soap are also used as leech repellents by the people all over the world. Except for soap others are not very common and not applied to the skin directly because they can be toxic to people. According to a social survey carried out it is found that people in Sri Lanka use Siddhalepa balm, Dettol, and medicinal oils [7] too to repel leeches. Compared to all the above-mentioned repellents, it is found that the leech socks sprayed with a repellent such as tobacco [3][7] and betel [7] is the most effective method that is used. This is more similar to the 'leech garters' used by Europeans in Kandy, Sri Lanka in 19th century[8] and this prevents leeches encountering the skin to some extent.

None of these methods have demonstrated totally satisfactory results and a need of a proper leech repellent, which is not toxic to people and with high persistence has become an issue in the leech infested areas.

Plants that have shown repellent activity against leeches during leech studies.

According to the studies that have been carried out it has been shown that plant extracts of *Nicotiana tabacum* L.(tobacco).[7], *Corymbria citriodora* (Hook.) (lemon eucalyptus)[8], *Sapindus rarak* DC. (soapberries), *Catunaregam spathulifolia* Tirveng., *Vernonia elaeagnifolia* DC.[1], *Callistemon rigidus* R.Br. (Bottle Brush)[4], *Acorus calamus* L. (wada kaha)[8], *Ocimum* spp.[8], *Zanthoxylum armatum* DC. (timur)[8][4] and the essential oils of *Azadirachta indica* A.Juss (neem)[4][9] *Cymbopogon nardus* (L.) Rendle (citronella)[8][9] *Curcuma longa* L. (saffron)[8], *Cinnamomum* spp. (cinnamon)[8] have shown repellent activity against leeches.

Further it was observed from a social survey carried out that people in Sri Lanka use the water extracts of *Piper betle* leaves to treat the leech socks.

Plants that were used for the study.

The taxonomy of the plants used in the current study are given in the table 1.

Plant	Kingdom	Order	Family	Genus	Species
Tobacco	Plantae	Solanales	Solanaceae	*Nicotiana*	*N. tabacum*
Betel	Plantae	Piperales	Piperaceae	*Piper*	*P. betle*
Citronella	Plantae	Poales	Poaceae	*Cymbopogon*	*C. nardus*
Eucalyptus	Plantae	Myrtales	Myrtaceae	*Eucalyptus*	*E. globulus*
Neem	Plantae	Sapindales	Meliaceae	*Azadirachta*	*A. indica*

Table 1: Taxonomy of plants used in the study.

Features of the family Haemadipsidae

Figure 1: A land leech

Kingdom	:	Animalia
Subkingdom	:	Eumetazoa
Phylum	:	Annelida
Class	:	Clitellata
Subclass	:	Hirudinia
Family	:	Haemadipsidae

Leeches are segmented worms and are closely related to the earthworms. There are nearly 500 species identified by scientists. Leeches cannot be categorized as parasites because only very few of them show the real parasitic behaviour. Neither are all the leeches blood suckers; they are rather highly evolved predators on smaller invertebrates such as insects, crustaceans, and annelids. The prey animals are sucked in and swallowed whole. The leeches that belong to the family haemadipsidae are blood suckers, which are well-known.[2] Leeches can vary in size from 7 mm to 200 mm.

Blood-sucking leeches can be divided into three categories depending on their preferred habitat;(1) aquatic leeches (Hirudidae), inhabit fishing ponds and rice paddies;(2) nasal leeches (Praobdellidae), known to enter the nasal cavities of dogs, pigs, buffaloes, and even humans;(3) terrestrial blood-sucking leeches/land leeches (Haemadipsidae), which can be seen in humid, shaded forest floors.[1]

Leech body is divided into 34 segments and contains two suckers, one at each end: anterior sucker and posterior sucker. Anterior sucker is formed with the first 6 segments of their body, and it is used to connect to the host, while posterior sucker is mainly used to stay attached to the prey. [10] Leeches belong to family Haedipsidae are also known as "jawed land leech" because they contain two or three jaws in their anterior sucker.[11]

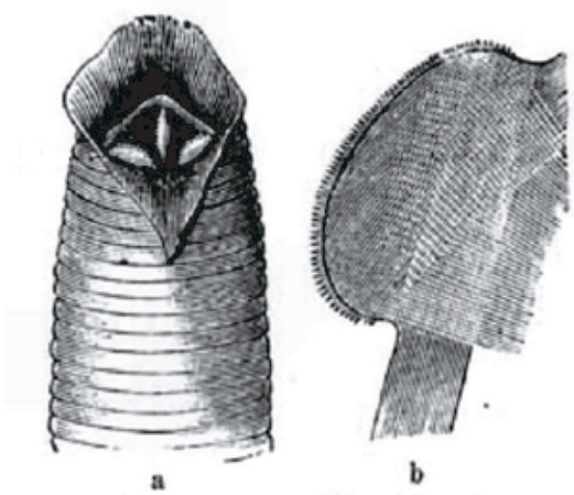


Figure 2:
(a) anterior sucker with jaws,
(b) posterior sucker

Out of thirty-four segments first six segments have been identified as head segments which include anterior sucker and the brain. This part is followed by twenty-one mid body segments which contain twenty-one neuronal ganglia, reproductive organs and nine pairs of testes[12], which indicates that the leeches are hermaphrodites (contain both sex organs)[6] the last seven segments are fused to form the posterior sucker as well as its posterior brain. Leeches also have a brain in each segment which concludes that they have a total of thirty-four brains in their body. [12] Adult leeches reproduce once or twice and then die.[6]

Leeches use both suckers to travel swiftly to the host's body and once they find a shadowy,

protected spot, they cut a hole in the host with its sharp teeth. Then they release an anaesthetic with their saliva to numb the skin and keep the host unaware of it. Then they release an anticoagulant called 'hirudin' to stop blood clotting and keep it flowing as long as the leech is feeding. This anticoagulant also stops the host's blood clotting inside the leech's gut, hence makes it easy to digest. Leeches can consume blood nearly five times of their bodyweight and after they are full of blood, they drop off. After a good meal, they can survive for months.[6]

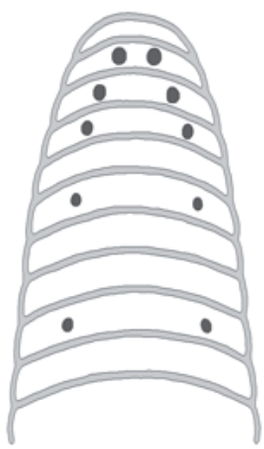


Figure 3: Placement of eyes of a leech

Leeches have five pairs of eyes with the last two pairs being separated by two eyeless segments.[11] Also the body of the leech is covered with well-developed receptors which are called as chemoreceptors.[13][6] Therefore they can feel warm blooded animals by sight, temperature change, vibrations and smell. Also they can sense the carbon dioxide in the air, which indicates that there is a living animal nearby. Leeches wave its head and body around in order to gain signals about its surrounding.[6] When there is a repellent around it can sense it and tries to go in a different direction avoiding the repellent. The chemoreceptors give information about the substances the anterior sucker comes in to contact, and the substances that the blood running through buccal cavity contains.

Different repellents act in different ways on leeches, but most of them affect their muscles. Some repellents increase or decrease tonic and rhythmic contractions of the muscles, some make it hard to move the muscles and some kill the leeches on the spot.[13]

Objectives of the study

- Preparation of the plant extracts and testing each one for the repellent activity by carrying out a laboratory test.
- Identification of a suitable mixture of the plant extracts
- Development of a leech repelling product

2. MATERIALS AND METHODS

Plant extracts

Materials

Extracts of the leaves of *Piper betle* (870 g) and *Nicotiana tabacum* (695 g) were prepared as follows.

The leaves were chopped into small pieces and placed in five 500 mL conical flasks. Ethanol (80%) (450 mL) was added to each conical flask. The flasks were kept in the dark for a week. Flasks were shaken occasionally during this time to ensure maximum extraction. Two extractions were carried out for each plant type.

At the end of the extraction, the solvent in betel extract became dark green in colour and leaves were light yellow. Tobacco extract was dark brown in colour while the leaves retained its original brown color.

Each extract was filtered and concentrated using the rotary evaporator. Temperature of the water bath was maintained at 55-60 °C. The solvent was recovered and reused for the second extraction of the same plant type.

Dry weights of the samples were taken and stored in the refrigerator till they are taken for testing.

Nicotiana tabacum dry weight = 180.33 g (26 %)
Piper betle dry weight = 31.64 g (3.6 %)

Essential oils of *Cymbopogon nardus* (citronella oil), *Eucalyptus globulus* (eucalyptus oil) and the seed oil of *Azadirachta indica* were purchased from reliable sources.

Preparation of the test solutions

Solvent : 45.9% ethanol, 19.6% isopropanol, 34.5% distilled water.

Each extract and the commercially purchased oils were dissolved in the solvent and the suitable concentration for each was identified separately by carrying out a laboratory test. Two concentrations were found for each repellent; the minimum concentration at which the leeches do not cross the paper ring at all and the maximum concentration at which the leeches cross the paper ring with difficulty. Test solutions were prepared with the above-mentioned concentrations to carry out the laboratory test.

By varying the concentration of each repellent in a mixture of the two most effective repellents, the most effective mixture to repel leeches was identified after several laboratory tests. A solution mixture containing 0.8% (w/v) of tobacco extract and 10% (v/v %) citronella oil was prepared as the final solution to treat the socks to be used at the field trial.

Laboratory test for the effectiveness of each repellent

The laboratory test was done with filter paper rings treated with each test solution. The outer diameter of each ring is 20 cm, with 6 cm diameter hole in the middle. These rings were treated with the test solutions one at a time until they are saturated with the solution. Spraying was done inside a fume-hood and the excess repellent was allowed to drain. Then the saturated rings were placed on a white tile and then a circular piece of untreated filter paper disc, 5.5 cm in diameter was placed in the centre of each ring. Therefore, the diameter of the filter paper disc was 0.5 cm smaller than that of the hole in the ring. The filter paper disc in the centre was moistened with distilled water before a leech was placed on it. Then a single leech was kept in the centre of the untreated filter paper disc and its behaviour was observed for 20 minutes. The experimental set up is shown in figure 4.

Test was carried out with untreated dry paper rings as controls and another set of tests were carried out to test the effect of the solvent on leeches. Fifteen treatments for each plant extract and essential oil were carried out after identifying the suitable concentrations by preliminary studies carried out. Neither paper rings nor leeches were reused during the experiment. Leeches for the test were collected from Kalutara, Avissawella, Deraniyagala and Theldeniya areas 3 or 4 days before the tests were done.

For each repellent minimum concentration at which the leeches do not cross the paper ring at all and the maximum concentration at which the leeches cross the paper ring with difficulty was observed. The crossings in to the filter paper ring and out of the ring to the other side were recorded as 'successful crossings' and the time taken for each successful crossing was noted. The crossings into the paper ring in which the leeches either returned to the inner filter paper disc or became immobilized and died on the ring was recorded as 'failed crossings'. In addition to that, the number of attempts taken to enter the treated ring was also observed for up to 10 minutes and it was recorded as 'touches with the oral sucker'.[8]

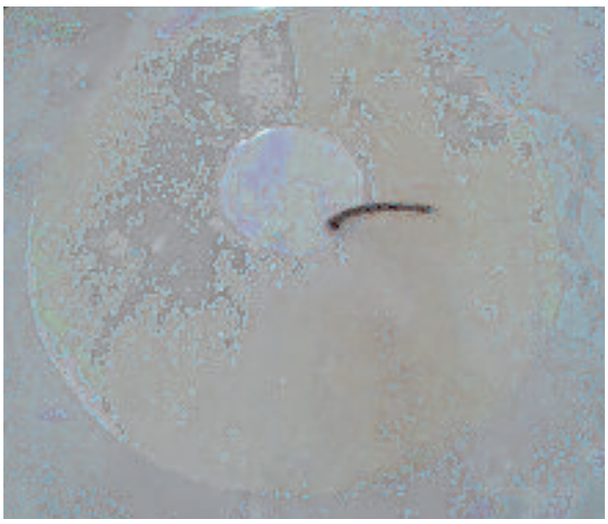


Figure:4
Experimental set up for evaluating the repellent activity of plant extract

Field evaluation of repellent activity repellents activity

From the studies carried out it was shown that the extracts of tobacco and citronella were the most effective out of the five plant materials. A test solution for field evaluation of repellent activity was prepared using 0.8% of tobacco extract and 10% citronella oil.

Field Test

The field trials were conducted in a rubber estate at Nivithigala in Ratnapura area for seven consecutive days from 7.00 am to 8.00 am to evaluate the efficiency and the persistence of the repellent mixture on socks. Four pairs of socks were impregnated with the repellent mixture 24 h prior to the trials and they were separately dried, away from direct sunlight and strong ventilation and stored in separate polyethylene bags till they were used for the field test. Five pairs of socks, four treated and one control, were given to 5 workers, who volunteered to walk in the leech infested foot track for 10 minutes. The sixth participant without socks was asked to walk in the front with the intention of arousing the leeches.[1],[4],[8]

The experiment had to be carried out during the early hours of the day in month of June and the relative humidity and the temperature of the area was recorded to be 67% to 72% and 25 °C to 27 °C[27] respectively. Leeches remain inactive during heavy rains and hot climate but become active during light drizzle. The moist and humid climate favour the leech infestation.[4]

Figure 5:
Volunteers wearing the socks treated with the repellent mixture.

Figure 6:
Foot-track where the field trials were carried out.

3. RESULTS

The minimum concentration at which the leeches do not cross the paper ring at all and the maximum concentration at which the leeches travel with difficulty are given in table 2.

Repellent	Minimum concentration at which leeches do not cross the paper ring at all	Minimum concentration at which leeches travel with difficulty
Nicotiana tabacum	2% (w/v %)	1% (w/v %)
Piper betle	50% (w/v %)	40% (w/v %)
Cymbopogon nardus	20% (v/v %)	15% (v/v %)
Eucalyptus globulus	60% (v/v %)	50% (v/v %)
Azadirachta indica	No repellent effect was observed against leeches.	

Table 2: Repellent activity of plant extracts

It was important to find out the minimum concentration required to repel the leeches completely in order to find out the potential repellent activity of each substance that was tested. Tobacco extract exhibited a high potential to repel leeches at a very low concentration of about 1 % (w/v%). Though some leeches crossed the treated filter paper successfully, but with difficulty at 1% tobacco level, at 2% (w/v %) concentration they could not cross the ring at all. Ones who entered the ring ended up dying. Citronella showed the minimum repellent activity at 15% (v/v %) which claimed to be the second highest. Also it contained a strong smell. Betel which exhibited the repellent activity at 40% (w/v %) concentration gave a dark green-brown colour solution but with no significant smell. Eucalyptus oil, which was expected to show the repellency at a lower concentration due to its strong odour, showed the minimum repellent activity at 50% (v/v %). The essential oil is highly volatile therefore it evaporated off quickly.

Neem oil did not show any repellent activity at all.

Most of the leeches used for the tests with citronella and tobacco, even the ones that crossed the rings with difficulty, died after the test. Whereas the number of deaths recorded for other repellents were lower compared to them.

Statistical analysis of the results of the laboratory test was done using 'analysis of variance (ANOVA). Spray levels, observation time and the other factors were kept fixed whereas the types of plant materials were considered as the independent factor.

ANOVA: SINGLE FACTOR

SUMMARY					
Groups	**Count**	**Sum**	**Average**	**Variance**	**Std. Deviation**
Control	15	2775.66	185.044	10265.7354	101.3199654
Solvent	15	1591.23	106.082	8702.29646	93.28610004
Tobacco extract 1%	15	1220.23	81.34866667	10103.21444	100.5147474
Citronella oil 15%	15	680.38	45.35866667	13364.8119	115.6062797
Betel extract 40%	15	2252.36	150.1573333	48606.91615	220.4697624
Eucalyptus oil 50%	15	890.47	59.36466667	11854.33263	108.8776039

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	219679.3434	5	43935.86869	2.561925281	0.033036021	2.323126498
Within Groups	1440562.298	84	17149.55116			
Total	1660241.641	89				

Table 3: ANOVA calculation for the laboratory test results- time measurements

Single factor ANOVA calculation was done to analyse the data at 5% significant level (95% confidence level), and it proved that there is a significant difference in the time measurements (time taken to cross the rings successfully) between the samples.

Neem oil failed to show any repellent activity towards leeches. Therefore, it was not considered for the analysis.

The behaviour of the leeches during the laboratory test is given in figure 7.

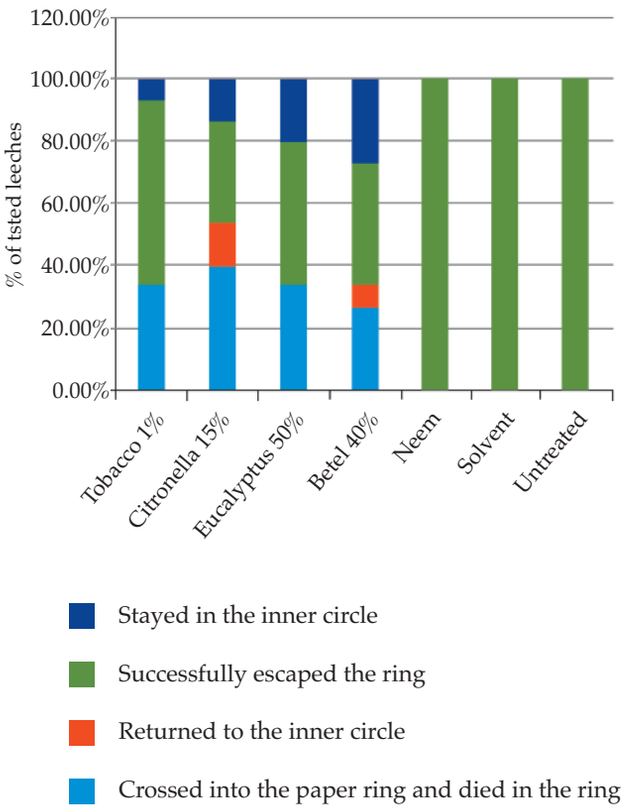


Figure 7: The behaviours of the leeches for different types of treatments.

Figure 7 shows the percentages of leeches that succeeded or failed in crossing the paper rings treated with different plant materials, solvent and the untreated papers. Solvent and the untreated paper ring showed 100% successful crossings. This implies that the solvent or the untreated filter papers do not exhibit a large effect on leeches. Tobacco showed the highest number of successful crossings (60%), but the concentration used was very low (1%) whereas citronella showed the lowest number of successful crossings (33.33%) with a concentration of 15%. Citronella recorded the highest number of returns to the inner circle as well as deaths after entering the treated paper. Tobacco and eucalyptus did not record any returns to the inner circle at all. Betel recorded the highest number of leeches that stayed in the inner circle after several attempts to enter the treated paper. Neem oil did not exhibit any repellent activity at all and 100% successful crossings were observed.

The field test was carried out one day after spraying the stockings with the spray formulation which was a mixture of the 0.8% tobacco and 10% citronella. The percentage repellencies were calculated using the equation given below.

$$= 1 - \left[\frac{\text{Number of leeches on treated stockings}}{\text{Number of untreated stockings}} \right] X100$$

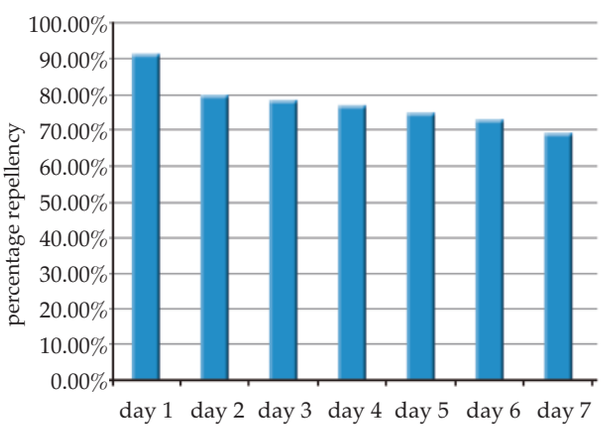


Figure 8: Daily leech repellency of the tested repellent mixture

One day after the stockings were impregnated with the repellent mixture it showed 91.667% protection against the leeches. As the number of days increased the percentage repellency decreased gradually (figure 8). Though the number of leeches that came to the stockings increased, no leech bites were recorded. This means even after seven days the repellent was strong enough to keep the leeches out from entering the feet.

4. DISCUSSION

A literature survey and a social survey was carried out prior to the research study in order to obtain an idea about the leech repellents that

have been tested before for their repellent activity and the repellents which are popular among the people in leech infested areas. It is found out that many synthetic and plant-derived insect repellents are tested for their potential repellent activity against leeches by researchers. Plants like *Azadirachta indica* (neem), which is used as a bio-pesticide against many arthropods did not show any repellent activity against the leeches which are Annelids, whereas citronella which is a good insect repellent showed better repellent activity against leeches too. *Nicotiana tabacum* (tobacco) which is popular among the travellers and hikers also showed good repellent activity against leeches.

During the laboratory tests, time measurements varied in a long range in an unexpected manner. There can be several reasons for this behaviour. Time taken to cross the ring depended on the activeness of the leeches. It was observed that the activeness of the leeches is irrespective of their size. For a particular concentration of the repellent, while some big leeches remained in the inner ring after several attempts to enter the treated paper, small leeches crossed the ring successfully with a short period of time. Also the time taken to cross varied with the size of the leeches. Big leeches since they can stretch longer travelled across the paper rings faster while small ones have to stretch several times to cross the ring. As the time period between the day the leeches were collected and the day they were used for the laboratory test increased the activeness of the leeches decreased. Also they became quite inactive during day time than in the mornings or evenings, and sometimes the organic solvents that were used to prepare the solutions also have affected the time measurements.

It was difficult to find leeches of the same size and the same species to be used for the laboratory test due to lack of rain to the leech infested areas. Several laboratory tests have been carried out, by increasing the concentrations from a lower value, to find out the 'maximum concentration at which leeches travel with difficulty' and 'the minimum concentration that leeches do not cross the ring at all'. Two concentrations for each repellent were obtained. Citronella and eucalyptus has strong smells while betel and tobaccos do not give a noticeable smell at all. But it was observed that the repellent activity did not depend much on the strength of the smell. Leeches got repelled after they touched the treated ring with their anterior sucker. Once they entered the ring, at the above mentioned concentrations, most of them could not move their bodies easily specially the posterior sucker, and those who escaped seemed damaged. At high concentrations leeches died as they entered the ring, by the repellent becoming toxic.

It is possible that the person who was carrying out the laboratory test acted as a stimulus to the leeches in the ring. It was assumed that the leech will feel the changes in the CO_2 levels in the atmosphere, which will make it feel that there is a human nearby, so that it will try to move from the ring.

During the field trial it was noticed that the leeches came and touched the stockings and then they went away. The ones, who climbed up on the stockings, curled up and fell down half away. No side effects such as itching, burning or any other skin problems were recorded from the volunteers after using the stockings.

5. CONCLUSION

Considering all the plant extracts and the essential oils used, *Nicotiana tabacum* (1%) showed the highest repellency against leeches followed by *Cymbopogon nardus* (15%), *Piper betle* (40%), *Eucalyptus globulus* (50%) respectively. *Azadirachta indica* did not show repellent activity towards leeches.

The final product, which is a mixture of *Nicotiana tabacum* (0.8%) and *Cymbopogon nardus* (10%)

showed high repellent activity towards leeches during the field trials carried out. Persistence of the product was high on the stockings and therefore percentage repellency of the final product was high.

Therefore, this product can be developed as a herbal leech repellent by further studies.

6. REFERENCES

[1] Vongsombath,C; de Boer, H.J.; Palsson, K. Keeping leeches at bay; Field evaluation of plant derived extracts against terrestrial blood sucking leeches in Lao PDR Acta Tropica. 2011,119, 178-182

[2] Ramel, G. The Hirudinae http://www.earthlife.net/inverts/hirudinas.html (accessed 8th June 2014)

[3] Leeches! Remove and Prevent. http://calvincho.hubpages.com/hub/Leeches accessed 09th Feb.2013

[4] Nath D.R.; Das N.G.; Das S.C. Bio repellents for leeches. Defence Science Journal 2002; Vol.52 pp73-76

[5] Koss,A. et al Am. J. Emerg. Med. 2008 (9).

[6] Tropical facts sheets. Leeches_Wet Tropics Management authority and the Queensland Environmental Protection Agency; Martin,S Tropical Topics (accessed 24th May 2014)

[7] Leeches! Preventive socks, Repellents and Treatments, Lakdasun, http://www.lakdasun.org/tools-tips/leeches-preventivesocks-repellents-treatments.ht (accessed 26th Dec.2012)

[8] Kirton, L.G. Laboratory and field tests of the effectiveness of the Lemon-eucalyptus extract, Citriodiol as a repellent against land leeches. Annals of Tropical Medicine & Parasitology. 2005 Vol 99, No.7 695-714

[9] Maia, M.F.; Moore, S.J. Plant based insect repellents: A review of their efficacy, development and testing, Malaria Journal 2011, 10 (Suppl l): S11

[10] http://www.newworldencyclopedia.org/entry/Leech (accessed 10th June 2014

[11] http://wikipedia.org/wiki/Haemadipsidae (accessed 10th June 2014

[12] http://en.wikipedia.org/wiki/Leech (accessed June 8th 2014)

[13] Veazie,W.H. Overbeck, R.C.; State of the Art study on the leech repellents. Advanced Research Project Agrncy 1963

[14] http://www.citylocal.net/lk/sabaragamuwa/ratnapura/weather-in-ratnapura (accessed June 20th 2014)

ARTHRITIS - PERSPECTIVE FROM MODERN WESTERN MEDICINE (I)

Jennifer Perera, MBBS, MD, Emeritus Professor, University of Colombo

Arthritis is inflammation of one or more joints in the body and is a leading cause of disability in the world.

As arthritis affects the quality of life of a person significantly, it is considered an important disease entity. The theory of pathophysiology of arthritis differ markedly between traditional and modern medicine which in turn guides diagnosis and management.

Historically, modern medicine evolved in Europe and specifically in Greece and Roman empire during the renaissance. The term “arthritis” is derived from the Greek words arthro-, meaning "joint," and -itis, meaning “inflammation. It affects both adults and children. The main symptoms of arthritis are joint pain and stiffness, which typically worsen with age. Joint pain occur due to pathological processes that affect ligaments, cartilage, bursae, tendons and bones surrounding the joint. When cartilage of the joint is damaged it leads to instability and loss of function of the affected joint.

Joints in the body are classified in to large joints and small joints. Examples for large joints are shoulder joint, elbow joint, hip joint, and knee joint. Wrist joint, ankle joint, joints in fingers and toes are considered as small joints. This classification is clinically useful to diagnose the type of arthritis because different arthritides have different patterns of joint involvement. Treatments vary depending on the type of arthritis. The main goals of arthritis treatments are to reduce symptoms and improve quality of life.

Modern Western Medicine describes several distinct types of arthritis. They are often classified as

* Inflammatory types of arthritis
* Non-inflammatory types of arthritis
* Septic arthritis

Examples of inflammatory type of arthritis are rheumatoid arthritis, arthritis which is linked to the dermatological condition psoriasis, ankylosing spondylitis (which mainly affects the spine) and arthritis associated with systemic lupus erythematosis (SLE). A common example of a non-inflammatory type of arthritis is osteoarthritis. Septic arthritis is caused when an infectious agent enter the joint causing inflammation. Most common types of arthritis are osteoarthritis and rheumatoid arthritis.

The predisposing causes of arthritis are many. Ageing, obesity, and trauma are contributory factors leading to development of osteoarthritis. Arthritis can also occur as part of a systemic disorder like SLE and psoriasis. Genetic factors too play a role. Therefore, many factors contribute to the development of arthritis in a person.

Different types of arthritis show varying clinical courses. They can either occur as a self-limiting illness or progress slowly impairing joint function ending with permanent disability. Some manifest as a relapsing and remitting illness. Joint pain, joint swelling, stiffness, decreased range of motion and redness will occur in most patients and are common to all types of arthritis.

Arthritis is mainly a clinical diagnosis. The X-ray of the joint will be useful in differentiating types of arthritis and excluding any trauma related factors. Arthritides such as rheumatoid arthritis and psoriatic arthritis will show changes in blood chemistry which can be identified by laboratory investigations. There is a place for ultrasound and MRI scans when there are diagnostic dilemmas.

Management depends on the type of arthritis affecting a person. There are numerous medical and surgical interventions available to treat this condition. Early commencement of treatment is very important in many types of arthritis to prevent joint deformity and permanent disability. In addition, life style and behavioural changes will prevent the development of lasting disability and plays a crucial role in improving the quality of life.

ARTHRITIS – PERSPECTIVE FROM AYURVEDA (I)

Dr. J.T.R. Jayakody, D.S.A.C.(Hon), MD (Ayur), Senior Consultant in Ayurveda, Link Natural Products (Pvt) Ltd

Diseases concerning joints (Arthropathy) occupy a prominent position in the medical history of mankind. Diseases of joints which can arise due to various causes result in bodily discomfort and compromises daily activities, as well as cause mental distress. Diseases in various joints can occur with common characteristics as well as more specific characteristics depending on the location of the joint. The tendency to develop joint diseases can be caused by trauma due to accidents or changes in the internal physiological processes or external causes. It needs to be recognized that aging is a fundamental factor influencing many diseases of joints.

In modern times, common problems related to joints have become a distressing feature that people of all age groups have to face. Although children, youth, adults and the elderly, all succumb to joint problems caused by various factors, the majority affected are adults and the elderly. It is seen that they are either those with long standing unresolved joint problems or weakness in joints due to ageing. Diseases which involve joints resulting in difficulties in carrying out day to day activities, which may be associated with restricted movements, pain, swelling, redness and feeling of heat are categorized as Arthropathy. The condition identified as inflammatory arthritis in modern western medicine is identified in Ayurveda also as an inflammatory (classical term "Pradaha") condition of the tissues of the joints.

Ayurvedic texts written at different time periods have discussed joint diseases in different ways. Different conceptual frameworks have led to joint diseases being classified in various ways. A knowledge of the causes of different types of joint diseases and associated complications and treatment methods can be obtained by studying these texts. Thus, a study of the ancient fundamental texts such as "Charaka Samhita" and "Sushruta Samhita" shows that joint diseases are categorized as "Vata Roga".

LINK NATURAL SWASTHA AMURTHA IN SRI LANKA

Anupamanie Uthpalawanna Sri Narayana

"Swastha Amurtha" was conceived as a wellness drink after an internal scientific study in the research and development centre of Link Natural. The Product was launched on the 2nd of April 2018 at the Taj Samudra Hotel. Link Natural "Swastha Amurtha' is manufactured using 100% natural Nelli (*Phyllanthus emblica*) and Rasakinda (*Tinospora cordifolia*). Successful results of consuming this Nelli-Rasakinda herbal concoction was evident in ancient Ayurveda. By combining the wisdom of ancient ayurveda with modern science and technology, the pure spirit, or the extract of this dry Nelli and fresh Rasakinda ingredients were taken carefully and accurately according to a standard operating procedure to manufacture a standardized formulation under the brand name “Swastha Amurtha”. The product is manufactured in a HACCP, ISO 9001, ISO 14001 and GMP Certified production facility.

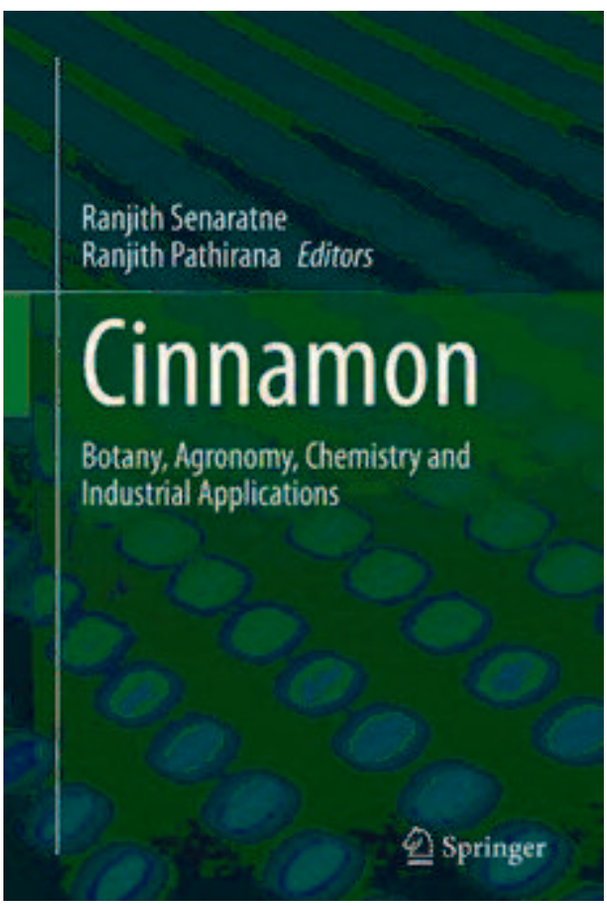
"Swastha Amurtha" is formulated as free-flowing granules, which are easily soluble in water. When these granules are dissolved in water, a dark brown colored turbid solution is formed. "Swastha Amurtha" can be prepared using either hot or cold water according to the consumer’s preference. “Swastha Amurtha” helps to relieve conditions such as prickly heat, rough or dry skin conditions and acne, discomfort due to burning sensation in heels, body and eyes, heel fissures, discomfort due to gastritis and burning sensation

in the stomach, discomfort due to excessive sweating and body odor, and discomfort due to burning sensation during urination. According to Ayurveda the main reason for the occurrence of these common problems is that impurities get accumulated in the blood after the metabolic processes in the body. If the toxic matter or the metabolic waste is not excreted from the body properly, they get accumulated inside the body, causing various discomforts. Our lifestyle has changed due to our busy schedules where we do not get much time to think about our health, no matter how important it is. We do not have time for regular exercise, and we tend to consume more instant and fast food even though the health risks are known. Due to this unhealthy and sedentary lifestyle, the impurities produced in the body are not optimally excreted. This causes heaty body conditions, also and affect the immune system.

So, to boost our immunity, to purify our blood and to cool our body we need a convenient method. In ancient times, our ancestors used natural ingredients to bring about these effects. Nelli and Rasakinda were combined in a water based drink as a Kasaaya prepared on the principles of Ayurveda. However, in today's busy world there are many challenges in selecting the correct, authenticated, high quality ingredients. Due to busy lifestyles of most people, they have no time to visit ayurveda pharmacies, buy the ingredients, prepare it according to ayurveda principles and consume at the right frequency. Link Natural innovated a solution to all these problems in the form of Link Natural "Swastha Amurtha" which is a 100% natural herbal drink that can be easily prepared in less than a minute. The raw materials which are used to manufacture the product are of the highest quality and standard, verified by scientific processes using the latest technology. Daily usage of "Swastha Amurtha" purifies the blood, cools the body, and boosts the immunity. As a result of these actions, it helps to resolve many discomforts which can be seen in our day-to-day life. In short, "Swastha Amurtha" helps to live a healthy and long life.

NEW ADDITIONS TO THE LINK LIBRARY

CINNAMON: BOTANY, AGRONOMY, CHEMISTRY, AND INDUSTRIAL APPLICATIONS



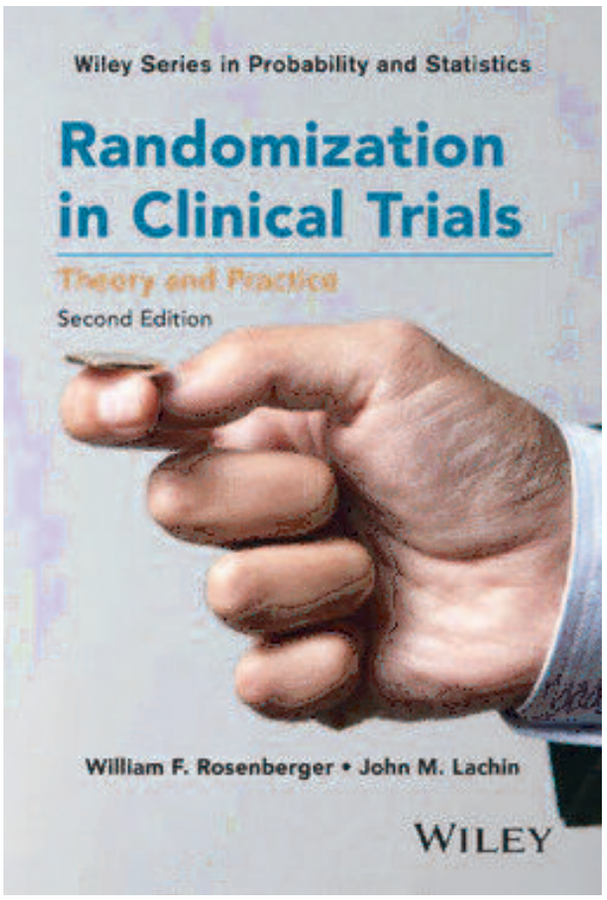
First book ever published on True Cinnamon (*Cinnamomum zeylanicum* Blume; synonym (J. Presl.) dealing comprehensively with Cinnamomum verum the latest advances of its botany, genetics and biotechnology, agronomy, chemistry, pharmacology, and industrial applications

Chapters on historical and ethno-botanical aspects of cinnamon trade will be of particular interest to readers that enjoy the history and commerce of food science.

Specific focus on the molecular characterization, genetic barcoding, and chemical profiling of True Cinnamon. These studies differentiate True Cinnamon from Cassia Cinnamon; the latter contains high content of coumarin which is carcinogenic.

- Authors: Ranjith Senaratne, Ranjith Pathirana
- Publisher: Springer
- ISBN: 978-3030-544-25-6
- Hard cover: 442 pages
- Year: January 26, 2021

RANDOMIZATION IN CLINICAL TRIALS: THEORY AND PRACTICE



* Authors: William F. Rosenberger, John M. Lachin
* Publisher: Wiley & Sons, Incorporated, John
* ISBN: 978-1-118-74224-2
* Hard cover: 288 pages
* Year: November 2015

Featuring a unique combination of the applied aspects of randomization in clinical trials with a nonparametric approach to inference, Randomization in Clinical Trials: Theory and Practice, Second Edition is the go-to guide for biostatisticians and pharmaceutical industry statisticians.

Randomization in Clinical Trials: Theory and Practice, Second Edition features:

- Discussions on current philosophies, controversies, and new developments in the increasingly important role of randomization techniques in clinical trials
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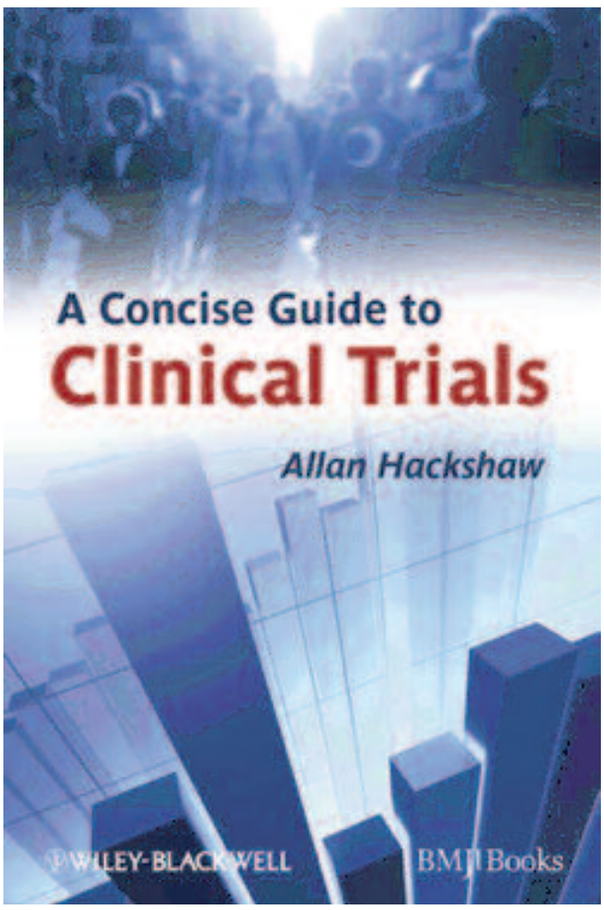
Randomization in Clinical Trials: Theory and Practice, Second Edition is an excellent reference for researchers as well as applied statisticians and biostatisticians. The Second Edition is also an ideal textbook for upper-undergraduate and graduate-level courses in biostatistics and applied statistics.

A CONCISE GUIDE TO CLINICAL TRIALS



DESCRIPTION

Clinical trials have revolutionized the way disease is prevented, detected, and treated, and early death avoided, and they continue to be an expanding area of research. They are central to the work of pharmaceutical companies, and there are many academic and public sector organizations that conduct trials on a wide variety of interventions, including drugs, devices, surgical techniques, and changes in behavior and lifestyle.

A Concise Guide to Clinical Trials provides a comprehensive yet easy-to-read overview of the design, conduct and analysis of trials. It requires no prior knowledge on the subject as the important concepts are introduced throughout. There are chapters that distinguish between the different types of trials, and an introduction to systematic reviews, health-related quality of life and health economic evaluation. The book also covers the ethical and legal requirements in setting up a clinical trial due to an increase in governance responsibilities and regulations.

This practical guidebook is ideal for busy clinicians and other health professionals who do not have enough time to attend courses or search through extensive textbooks. It will help anyone involved in undertaking clinical research, or those reading about trials. The book is aimed at:

- Those wishing to learn about clinical trials for the first time, or as a quick reference guide, for example as part of a taught course on clinical trials
- Health professionals who wish to conduct their own trials, or participate in other people's studies
- People who work in pharmaceutical companies, grant funding organizations, or regulatory agencies

* Author: Allan Hackshaw

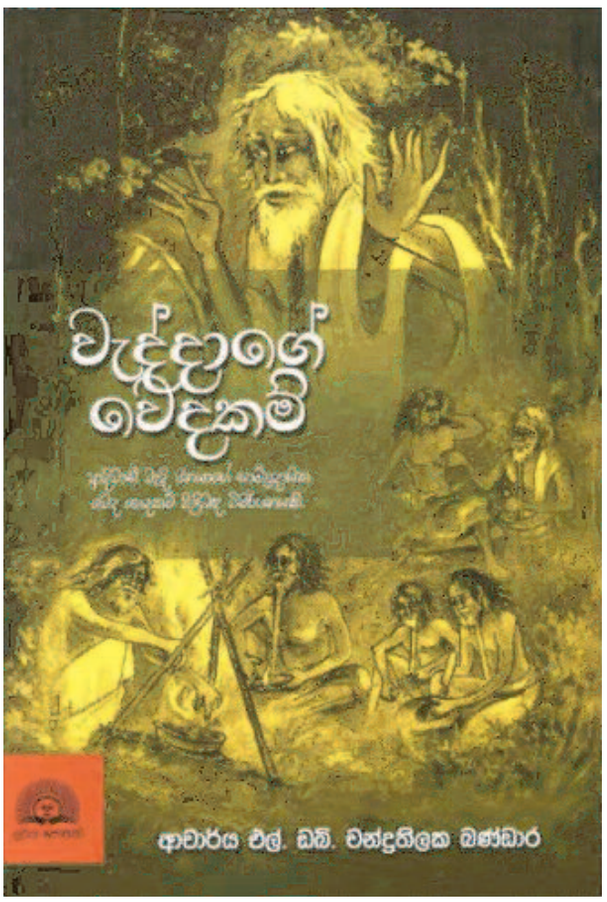
* Publisher: Wiley & Sons, Incorporated, John

* ISBN: 978-1-405-16774-1

* Hard cover: 226 pages

–Year: April 2009

WADDAGE WEDAKAM



An investigation into the traditional medicine of the indigenous Vedi people in Sri Lanka.

* Author: Dr. L.W. Chandrathilake Bandara
* Publisher: Sooriya Publishers, Maradana, Colombo 10
* ISBN: 978-955-6565-05-8
* Hard cover: 377 pages
* Year: 2019 (1st edition)

AVAILABILITY OF LINK PRODUCTS

The availability of Link Products in readily accessible outlets is indicated (✓) in the table given below.

	Super Market													Grocery	Phamacy	Osusal
	Sathosa	Cargills	Keels	Arpico	Laugfs	PDK	Health guard	Family super	Air Force	City Exchange	CIC	SPAR	Odel			
Link Samahan	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Link Enriched Paspanguwa	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Natural Sudantha	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Kesha Hair Oil	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Hair Care Cool	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Akalapalitha	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Swastha Thriphala		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Swastha Amurtha	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓
Link Five Herbs		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Gotukola Tea			✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Osupen		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Muscleguaed		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link SP Balm	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Link Essentials - Siddhartha Oil		✓		✓			✓					✓			✓	✓
Link Essentials - Pinda Oil		✓		✓			✓					✓			✓	✓
Link Essentials - Mahanarayana Oil		✓		✓			✓					✓			✓	✓
Link Essentials - Kendaperalumhara Oil		✓		✓			✓					✓			✓	✓
Link Essentials - Kolaseleshma Oil		✓		✓			✓					✓			✓	✓
Link Essentials - Sarvavisadee Oil		✓		✓			✓					✓			✓	✓
Link Essentials - Composite Pack		✓	✓	✓		✓		✓				✓			✓	✓
Link Dekatone		✓					✓					✓				✓
Link Viritone		✓					✓					✓				✓
Herbal Pharmaceutical														✓		✓

NOTE TO POTENTIAL CONTRIBUTORS

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The DIGEST is a popular publication, albeit a scientific one, dedicated to medicinal plants, herbal healthcare and personal care products, essential oils, aromatherapy, herbal therapy and Ayurveda, and related healthcare systems. It is published bi-annually.

The DIGEST welcomes contributions in English in the category of reviews, brief communications, ethno reports in brief, phytomedical and phytochemical communications, book reviews, and reports on safety and efficacy of phytomedicines.

Authors may submit manuscripts by email to :

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Editor-in-Chief
Link Natural Digest
library@linknaturalproducts.com

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