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Appendix A : Questionnaire

1. Questionnaire (English Version)

A. i. Do you know about trees/plants which could purify water? Yes/No

ii. If Yes, What are the species that you know and details?

iii. Do you know any studies carried out on above species? Yes/No

iv. If yes, please specify the details (study done by whom/organization and contact details)

v. How and for what can these trees could use for? (eg. town and country planning?)

B. i. Do you know about *TerminaliaArjuna* (Sinhala :Kumbuk, Tamil:Marutamaran)? Yes/No

ii If Yes, please specify

ii. Do you know any studies carried out on *TerminaliaArjuna*? Yes/No

iii. If yes, please specify the details (study done by whom/organization and contact details)

iv. Do you know locations of *TerminaliaArjuna*near a natural water resource? Yes/No

v. If yes, please specify

C. i. Can Kumbuk Tree grow as a plantation? Yes/No

ii. If Yes, Reason/Benefits :

iii. If No, Reason:

D. i. Is there any information/recommendations relevant to above that you could share with? Yes/No.

ii. If yes, please state

2. Questionnaire in Local Language (Sinhala)

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A. i. ජලය පිරිසිදු කරන පැල හෝ ශාක පිළිබඳව ඔබ දන්නවාද? (ඔව් / නැත)

ii. ඔබෙ පිලිතුර "ඔව්" නම් එම වර්ග මොනවාද? ඒ පිළිබඳව විස්තර කරන්න.

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iii. මෙම ශාක පිලිබඳ කර ඇති අධ්‍යනයන් පිලිබඳ ඔබ දන්නවද? (ඔව් / නැත)

iv. ඔබෙ පිලිතුර "ඔව්" නම් ඒ පිළිබඳව විස්තර කරන්න. (ඒ පිළිබඳව තොරතුරු ඉදිරිපත්කල තැනැත්තෙක් හෝ ආයතනයක් හා සම්බන්ද විය හැකි මාර්ගයක් දක්වන්න.)

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v. මෙවැනි ශාක නගර හා රටවල් සැලසුම් කිරීමට යොදා ගන්නෙ කෙසේද?

B. i. ඔබ කුඹුක් ශාකය දන්නෙහිද? (ඔව් / නැත)

ii. ඔබෙ පිලිතුර "ඔව්" නම් ඒ පිළිබඳව විස්තරයක් ලබා දෙන්න.

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iii. ඔබ කුඹුක් ශාකය පිළිබඳ කර ඇති අධ්‍යනයන් පිලිබඳ ඔබ දන්නෙහිද? (ඔව් / නැත)

iv. ඔබෙ පිලිතුර "ඔව්" නම් ඒ පිළිබඳව විස්තර කරන්න. (ඒ පිළිබඳව තොරතුරු ඉදිරිපත්කල තැනැත්තෙක් හෝ ආයතනයක් හා සම්බන්ද විය හැකි මාර්ගයක් දක්වන්න.)

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v. කුඹුක් ගාක වැඩෙන ස්වභාවික ජලාශය ස්ථාන ඔබ දන්නවා ද? (ඔව් / නැත)

vi. ඔබේ පිලිතුර "ඔව්" නම් ඒ පිළිබඳව විස්තරයක් ලබා දෙන්න.

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C. i. කුඹුක් වගාවක් ලෙස ව්‍යාප්ත කල හැකිද? (ඔව් / නැත)

ii. ඔබේ පිලිතුර " ඔව් " නම් ඊට හේතු සහ ප්‍රයෝජන දක්වන්න.

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iii. ඔබේ පිලිතුර " නැත" නම් ඊට හේතු සපයන්න.

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D. i. ඉහත සඳහන් විස්තරවලට අදාල බෙදා ගත හැකි කොරකුරු ඔබ ලග තිබෙද?
(ඔව් / නැත)

ii. ඔබේ පිලිතුර "ඔව්" නම් ඒ පිළිබඳව විස්තරයක් ලබා දෙන්න.

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Appendix B : Research on Benefits of *Terminalia arjuna* and Reference Guide on Studies − Extracted from Examin.com Medical Disclaimer

Terminalia arjuna (Arjuna) also known asArjuna, Dhavala, Kakubha, Nadisarja, Veeravriksha, Partha, Indradru, is a tree that has its bark used medicinally, usually for the purposes of cardioprotection. It appears to reduce pressure and pulse rate, and may increase aerobic exercise capacity.

Terminalia arjuna (usually simply referred to as Arjuna) is a tree bark that is used medicinally in Ayurveda for the purposes of cardiovascular health pertaining to the heart itself. It has a large variety of bioactives, with the water extract showing promise at improving left ventricle function of the heart without any observable toxicity of side effects when taken at 500mg thrice a day (every 8 hours).

There are numerous human studies conducted on Arjuna bark, although many of them are low in sample size. Nevertheless, the water extract appears to be effective in improving cardiac function in persons who have recently undergone cardiac trauma or injury; Myocardial Infarction is the most commonly researched ailment in this regard. Only one study exists on otherwise healthy persons, but Arjuna showed benefit in improving left ventricle function in an exercise test and the benefits may affect a person regardless of health state.

In animal models, this extract appears to exert protection on cardiac tissue in response to various cardiac insults including beta(2)adrenergic agonists (like Ephedrine, although isoproterenol was used in the studies) and catecholamine's themselves.

The water extract appears to be effective for improving cardiovascular health, particularly at the level of left ventricle function. The studies in humans are underpowered at this moment in time and only one in healthy humans (preliminary evidence), but all evidence appears to be promising and in the same positive direction. The water extract appears to be quite safe.

Other extracts such as ethanolic or acetone, with different bioactives, may not have similar cardio protective effects (no human trials, but some *in vitro* evidence suggesting the bioactives are not in these extracts) yet appear to be somewhat cancer protective. Tumour growth in animal models is reduced with either the ethanolic

extract or isolated Arjunolic Acid (commonly seen as the main bioactive) as is reduced DNA damage in response to mutagens, and these are attributed to the antioxidative capacity of Arjuna which is comparable to Vitamin C on a per weight basis. Due to the anti-cancer effects of the ethanolic extract having some cytotoxic properties, and LD_{50} has actually been established with this extract and it is possible that side-effects may occur. Additionally, the anti-cancer evidence is somewhat limited as although cytotoxicity has been established in cancer cells a lack of evidence exists to assess healthy cells (a good anti-cancer drug will be highly selective in killing cancer cells, which Arjuna does, and not healthy cells, which Arjuna has not been sufficiently tested for).

Other possible uses of Arjuna include ulcer protection in the stomach with potency similar to Rantidine in one study (associated with the ethanolic extract), protection to the liver and kidney likely mediated by antioxidative properties (ethanolic extract) and the cardiovascular properties may increase anaerobic cardiovascular performance in healthy persons (with the one study using sprinting as a test) although this last claim has a lone study in support of it and no replication.

Ethanolic extracts have potent antioxidative and potentially potent anticancer effects, but although there are no reported side effects with the ethanolic extracts currently (due to a lack of human interventions) it is theoretically plausible that higher than recommended doses could be harmful related to the anticancer effects (cytotoxicity)

Things to Note

- The water extract (used for cardiovascular health) and ethanolic/acetone extracts (used for cancer prevention and antioxidant purposes) are markedly different and, for all intents and purposes, should be treated as different supplements

Goes Well With

- Ashwagandha (appears to benefit cardiovascular function on parameters that Arjuna does not, and combination therapy in health persons has at once been shown to confer additive benefits to exercise capacity with both being effective in isolation)

How to Take (recommended dosage, active amounts, other details)
A standard dose for the purposes of cardiac health appears to be 500mg of the bark (water extract) taken daily in the morning without food (no evidence exists to suggest that taking it *with* food is bad or anything). For persons who suffered cardiac trauma (such as Myocardial Infarction), this dose tends to be taken thrice a day every 8 hours

The leaf extracts and ethanolic extracts appear to be more related to the cytotoxic and anti-tumor effects, but not enough evidence exists to recommend an active dose of these extracts for human consumption.

Table of Contents:

3. Oral Cancer
4. Breast Cancer
5. Lung
6. Hepatocellular Carcinoma

10. Safety and Toxicity

Edit1. Sources and Composition

1.1. Sources

Terminalia arjuna (of the *Combretaceae* family) is a tree; a deciduous evergreen tree standing up to 20-30m above ground level.[1] The bark of the tree appears to have medicinal properties, mostly touted to be a cardioprotective agent.[2][3][4][5] It has been used in Ayurveda under the names of *Arjuna, Dhavala, Kakubha, Kumbuk, Nadisarja, Veeravriksha, Partha, and Indradru*.[6][7] Other notable species in the *Terminalia* family include *bellerica* and *chebula*.[8]

Beyond being used for its cardioprotective/cardiotonic abilities, Arjuna has also been reportedly used for genital health (leucorrhoea, Spermatorrhoea), urinary astringent, expectorant, and some Aphrodisiac properties.[7]

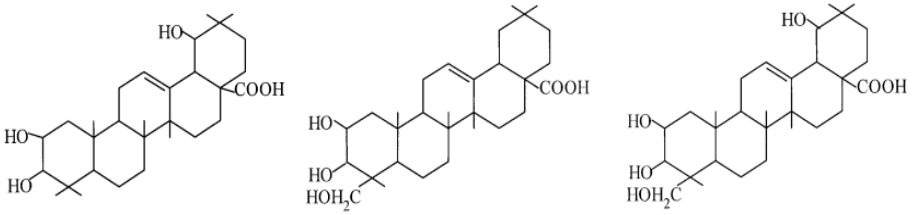
Traditional administration of Arjuna involves a decoction made with the bark and milk drank in the morning, or at least the powder of the tree bark; in the cases of fractures or contusions with echymosis, honey tends to be recommended.[9] Usually 1-3g of the bark is used per day.[10]

1.2. Composition

The tree bark, the main medicinal component, contains:

- The triterpenoids Arjunic acid and Arjunolic Acid,[11][7] up to 3% and 1.5% (respectively) of an Ethyl acetate extract or 2% and 0.9% of a diethyl ester extraction. Minimal quantities (0.2-0.3% of each) in acetone, methanol, and ethanol extracts with a 60:40 ethanol:water extract reaching up to 0.72% and 0.48%, respectively.[7] Arjunic acid has bioactive metabolites as well[12]
- Arjugenin and Arjunetin,[12] the former being an aglycone similar to Arjunic and Arjunolic Acids[13]
- Arjunasides A-E (Triterpenoid monoglycosides)[14] and Arjunetoside[15]
- Ajunglycosides IV and V in the butanolic/methanolic fractions,[16] also in the fruits of *Terminalia chebula*

- Arjunaphthanoloside, a napthanol glycoside[17]
- Termiarjunoside I and II (Oleanane Glycosides),[18] Terminoside A,[19] and Terminolitin[20]
- Pelargonidin, an anthocyanin[21]
- The cardenolide 16,17-dihydroneridienone 3-O-beta-D-glucopyranosyl-(1->6)-O-beta-D-galactopyranoside[22] and the structurally related xylopyranosyl(1->3)[23]
- Ursane triterpenoids (*2α,3β-dihydroxyurs-12,18-dien-28-oic acid 28-O-β-D-glucopyranosyl ester*)[24]
- Tannin structures (Gallic and Ellagic acids),[25] *3-O-methylellagic acid 4'-O-α-L-rhamnopyranoside*,[24] Casuarinin,[26] and Arjunin[27]
- (-)-epicatechin[24]
- Luteolin,[25] Quercetin, Kaempferol and Baicalein[21]
- Vitamin C at 1.47mg/100g (hydroalcoholic extract)[1]
- Vitamin E at 0.58mg/100g (hydroalcoholic extract)[1]



Arjunic Acid **Arjunolic Acid** **Arjugenin**

The three above triterpenoids are commonly seen as the main active ingredients, with other compounds with names related to this plant (Terminoside or Arjunoside as examples) tend to be glycosides of the three above aglycones. That being said, there is no evidence to support that these are the compounds that underlie the cardiovascular health properties although they appear to be causative of anti-cancer and anti-oxidative properties

And the fruits that the tree bears may contain:

- Arjunglucoside I-III and Arjunetin[28]

- Hydrolyzable tannin structures (Ellagic acid, Gallic acid, Corilagin, Chebulagic acid, etc.)[28]
- Chebuloside II and Bellericoside[28]
- Dietary minerals of Calcium, Magnesium, Zinc, and Copper[10]

Total phenolics may reach up to 8.05mg/100g in hydroalcoholic extracts of the bark; tannins may reach 5.1mg/100g.[1]

Edit2. Cardiovascular Health

2.1. Cardiac Tissue

In vitro, the water extract of Arjuna bark appears to have cardiotonic effects with 50ug/mL having similar potency (accessed via amplitude of CS (cell shortening), indicative of contractility) to 100nM Isoproteronol and 1mM Ouabain.[25] The mechanism appears to be different, as a decrease in the decay time during relaxation was noted with both Isoproterenol and Arjuna (not with Ouabain) and both Arjuna and Ouabain (not Isoproterenol) caused an increase in the rise time during contraction.[25] This was only observed with the water extract, with various organic extractions (ethanolic, ethyl acetate) causing varying effects and isolated Arjulinolic acid being arrhythmogenic (causing arrythmia).

Appears to have cardiotonic effects in vitro with isolated heart cells, seems to be associated with the water extract mostly

One month of treatment of 500mg/kg Arjuna bark extract (50% ethanolic) to normal and diabetic rats, reflex bradycardia (a reduction in heart rate in response to high blood pressure, which attempts to normalize blood pressure) was improved in the diabetic rats;[29] the state of diabetes being known to reduce this reflex.[30] The alterations in reflex tachycardia (an increase in heart rate in response to low blood pressure) were not improved by Arjuna over 30 days.[29] The beneficial effects on baroreflexivity has also been noted in isoproterenol (beta-2-adrenergic agonist; like Ephedrine) induced cardiac failure,[31]where Arjuna was able to exert rehabilitative and prophylaxtic protection and reduce both cardiac hypertrophy and fibrosis.

Reflex Bradycardia (Baroreflex) is a mechanism where the heart slows its pulsatile power when blood pressure gets too high, which acts to normalize blood pressure.

Arjuna, although the mechanisms are unknown, appears to preserve this reflex in situations where the reflex would be hindered (cardiotoxicity, diabetes). The opposite (reflex tachycardia) may not be influenced

In regards to cardiotoxicity, Arjuna appears to be protective against beta-2-adrenergic agonist induced cardiotoxicity (usually using isoproteronol as a means to induce toxicity) in the range of 100-200mg/kg water or water:ethanolic extracts in rodents;[31][32] this appears to extend to catecholamine-induced cardiotoxicity as well.[33]

Other studies in animal models have noted cardioprotection against sodium fluoride-induced oxidative stress,[34] the chemotherapeutic Doxorubicin,[35] ischemia-reperfusion,[36][37] and Carbon tetrafluoride.[38]

Appears to be protective against catecholamine and stimulant-induced cardiotoxicity after oral ingestion to rodents, and protective against other general stressors such as oxidative stress

Specifically looking at human interventions, in persons with myocardial infarction or ischaemic cardiomyopathy given 500mg of the bark extract after operation and for a subsequent 8 weeks in addition to standard therapy (relative to control, receiving only standard therapy of nitrates and aspirin/beta-blockers) noted an increase in left ventricular ejection fraction (12.32% versus 2.52%) and a reduction in left ventricle mass (20% versus no change) only in the Arjuna group without any observable toxicity.[39] Higher doses tend to be used, with *thrice* daily dosing of Arjuna bark water extracts showing benefit in persons with acute myocardial infarction (AMI) by reducing mitral regurgitations (independent risk factor for mortality in persons with AMI[40]) at 1 month (49%) and 3 months (72%) relative to baseline as assessed by echocardiography,[41] with improvements in diastolic function (E/A ratio) at 1 month (29%) and 3 months (48%).[42] 2 weeks of thrice daily dosing of 500mg Arjuna bark water extract alongside standard therapy noted improvements in left ventricle function including ejection fraction, and end diastolic and systolic volume (11 persons with AMI, 1 person with peripartum cardiomyopathy) and benefit to cardiovascular health remained when an open-label follow-up was conducted for 20-28 months.[43] The study recording mitral regurgitations[42] made note that a placebo group was given, but the placebo and its relations to active control were not elaborated on.

A trial with a larger sample (58 persons with chronic stable angina and provocable ischemia on a treadmill test) using 500mg Arjuna extract thrice daily against 40mg isosorbide mononitrate (vasodilating drug) as active control noted improved performance on a treadmill test (maximal cardiovascular exercise duration and recovery time) as well as cardiac function (maximal ST depression) where Arjuna was comparable to Isosorbide mononitrate.[44]

The thrice daily dosing of 500mg Arjuna water bark extract has also been used in one case study on Beta-Thalassemia where a reduction of Lp(a) from 51.8 to 39mg/dL was recorded;[45] the paper made note of a conference presentation (does not appear to be a publication) where a similar observation was noted.

In regards to human trials on cardiovascular health, a once (but more commonly thrice) daily dosing with even intervals between doses shows benefit to cardiovascular and left ventricle function in persons who have suffered myocardial infarctions, with the benefits appearing to extend to other cardiac conditions (Angina in one trial, Beta-Thalassemia according to a case study). Numerous studies, although many are used alongside standard cardiovascular drug treatment rather than monotherapy and most have small sample sizes and statistical power

2.2. Cholesterol and Lipids

In a research model of hypercholesterolemic rabbits the ethanolic extract of Arjuna (100-200mg/kg) over 72 days was able to attenuate the increases in triglycerides, LDL-C, HDL-C, and attenuate the increase in the artherogenic index by 49-80%; when compared to 10mg/kg Atorvastatin, 200mg/kg Arjuna tended to underperform nonsignificantly on all parameters.[46]

Triglyceride lowering effects have been noted in rats in response to Poloxamer 407, with the ethanolic outperforming the ethyl acetate fraction when 175-350mg/kg is taken alongside P-407.[47] P-407 is an experimental tool to assess high triglyceride levels by inhibiting LPL activity and preventing cellular uptake of triglycerides.[48] Another study using chemically induced high triglycerides (triton WR-1339) noted similar effects with only the ethanolic fraction being statistically significant, and was replicated in a diet-induced hypertriglyceridemia model where 250mg/kg of the ethanolic extract over one week.[49]

2.3. Endothelium

One study in smokers using Arjuna extracts at 500mg daily for 2 weeks noted that the impaired flow mediated vasodilation (FMD) seen in smokers is somewhat normalized with Arjuna, but not placebo; the authors attributed this to the antioxidative effects of Arjuna, as they are noted also with Vitamin C.[50]

Edit3. Interactions with Glucose Metabolism

3.1. Absorption

The 50% methanolic extract of the bark of Arjuna appears to be able to inhibit alpha-amylase with an IC_{50} of 302+/-0.55µg/mL, with the bioactives (currently unknown) possibly existing in the water component as the 100% methanolic extract has less inhibitory potential.[51]

3.2. Interventions

A study using the methanolic leaf extract in streptozotocin-induced diabetic mice noted that 100-200mg/kg bodyweight for 15 days was able to dose-dependently reduce elevated fasting blood glucose, with 100mg/kg attenuating 89% of the increase in blood glucose seen in diabetic control and both 200mg/kg Arjuna as well as the active control of 0.5mg/kg Glibenclamide both normalizing blood glucose.[52] Similar to the potency observed with glucose, both Arjuna at the higher dose and Glibenclamide drastically reduced serum liver enzymes and cholesterol to near control levels.[52]

Edit4. Interactions with Physical Performance

4.1. Interventions

A study assessing the effects of Arjuna (500mg water extract of the bark) with or without Ashwagandha (500mg water extract of the roots) in normal weight young adults over 8 weeks noted that, relative to placebo, maximal oxygen consumption capacity was increased 4.8% and average maximal power output (assessed by Sprinting and measured by a Kinematic Measuring System) increased 3.6% with a decrease of blood pressure from 123.00+/-2.87mmHg to 117.80+/-1.48mmHg (4.3%).[53]Pairing with Ashwagandha increased the power output to 11.6% with a 10% increase in relative power, and also induced significant improvements in VO2 max and oxygen carrying capacity (both seen with Ashawgandha in isolation).[53]

At least one study has noted improvements in anaerobic cardiovascular exercise performance (high intensity cardio), and the benefits appear to be somewhat additive with Ashwagandha

Edit5. Interactions with Hormones

5.1. Thyroid Hormones

An ethanolic extract of Arjuna at 21.42mg/kg and 42.84mg/kg in rats (human equivalents of 3.4 and 6.8mg/kg) who were administered thyroxine (T4, a thyroid hormone at 0.5mg/kg for two weeks) noted that the serum increase in T3 and T4 was attenuated by 42% and 79% by administration of Arjuna; the increase in lipid peroxidation of cardiac and liver tissue by T4 was simulatenously abolished.[54] Similar effects were noted in euthyroidic rats, but an increase in hepatic lipid peroxidation occurred.[54] The authors noted that similar effects with the antithyroid drug *Propyl thiouracil* and hypothesized that Arjuna ethanolic extract has anti-thyroid effects, and due to hyperthyroidism causing cardiac tissue enlargment[55][56] it is thought this may contribute to the cardioprotective effects observed with Arjuna.

Possible thyroid reducing properties, which need to be replicated with a water soluble extract (and mechanisms explored more). May possibly contribute to cardioprotective effects, although not likely to be the only mechanism (as cardioprotection has been noted in persons with normal thyroid status)

Edit6. Interactions with Organ Systems

6.1. Stomach

The methanolic extract of Arjuna appears to protect gastric tissue from lipopolysaccharide (LPS) from *Heliobactor Pylori*, which is known to induce ulcers.[57] This protective effect has been noted against Alcohol (7 day preload), Dexamethasone (10 day preload), and diclofenac sodium (single dose) where 100-200mg/kg of an 80% ethanolic extract conferred absolute protection against ulcer formation from Dexamethasone and alcohol, while 400mg/kg of an acute dose conferred absolute protection against Diclofenac Sodium.[58] The positive control in this study, Rantidine (35mg/kg Diclofenac, 50mg/kg alcohol, 4mg/kg Dexamethasone) also conferred absolute protection,[58] and these protective effects

against Diclofenac have been replicated with a methanolic extract and thought to be related to the anti-oxidative potential.[59]

6.2. Ears

One study noted that Arjuna extracts, especially acetonic extraction, was able to cause death of *Staphylococcus aureus*bacteria and extended to *Escherchia Coli*, *Acinetobacter sp*, *Proteus mirabilis*, and *Candida albicans*;[60] the authors hypothesized that the results suggest that Arjuna may be useful against ear infections (although admittedly preliminary).[60]

6.3. Liver

In isolated hepatocytes (HepG2), 5-100mcg/mL of the water extract of Arjuna is able to concentration dependently reduce the biomarkers of oxidation induced by TERT (pro-oxidative agent), with free radicals (ROS) and lipid peroxidation (TBARS) being reduced up to 69% and 62% respectively.[61] The reduction of antioxidant enzymes by TERT was also significantly attenuated up to 60% (SOD), 82% (Catalase), and Glutathione enzymes (62-65% for peroxidase and reductase; fully preventing any reduction in Glutathione S-transferase).[61] These antioxidant effects are thought to underlie results achieved with 50mg/kg water extract of Arjuna for 7 days prior to CCL_4 induced hepatotoxicity, where Arjuna significantly prevented the rise in GPT (dose dependent reductions up to 50mg/kg, no added benefit at 100mg/kg), normalized ALP, and increased hepatic levels of antioxidant levels either to control levels (Catalase and Glutathione S-Transferase) or above control (SOD), a potency similar toVitamin C at the same oral doses.[62]

6.4. Kidneys

250-500mg/kg of a hydroalcoholic extract of Arjuna bark (not an oral study, but the equivalent injected into perfused kidneys) appears to reduce oxidation and concomitantly increase antioxidant enzymes (Catalase and Glutathione).[1] A preservation of anti-oxidant enzymes in response to CCL_4 is observed to be of similar potency to Vitamin C[62] and has noted protective effects in the kidneys of Alloxan-induced diabetic rats at oral doses of 250-500mg/kg of the water extract.[63][64]

Molecules that are found in Arjuna that have been noted to exert protective effects on kidney cells include Casuarinin, a tannin structure, with more potency than the water soluble form of Vitamin E known as Trolox.[65]

6.5. Urinary Tract

In vitro, Arjuna appears to be able to reduce formation rates of calcium-based kidney stones (calcium oxalate and calcium phosphate tested) with the butanol extract being most effective.[66]

6.6. Testicles

One study using arsenic-induced testicular damage in rats noted that 4 days pre-treatment with isolated Arjunolic acid can prevent oxidative testicular damage and histological changes in response to arsenic.[67] This study noted that, *in vitro,* the antioxidant potential of Arjunolic Acid peaked at a concentrated of 0.4mg/mL and this appeared to correlated with an oral intake of 20mg/kg (as 50mg/kg did not confer additional protective benefits) and that the overall protective benefit was near absolute, and sligtly less effective than 100mg/kg Vitamin C.[67]

Edit7. Inflammation and Immunology

7.1. Mechanisms

One study has noted that Arjunic Acid and arjungenin, as well as their glycosides (Arjunetin and Arjunglucoside II) are able to scavenge free radicals without significantly influence superoxide release from polymorphonuclear immune cells.[68]

Edit8. Interactions with Oxidation

8.1. Mechanisms

Arjuna, particular Arjunic and Arjunolic acids, possesses anti-oxidative properties directly and in a DPPH assay (*in vitro* assay of antioxidative potential) has more potency than Vitamin C[69] or at least comparable at the same concentrations.[62] This is thought to underlie various protective effects of Arjuna in response to compounds with toxic effects mediated by oxidation such as Adriamycin[70] cadmium,[71] and arsenic.[72] Despite the aforementioned study showing more potency from Arjunolic relative to Vitamin C, studies that compare the two in living systems either note

similar protectiv effects[72] or more from Vitamin C (although this latter study used a much higher dose of Vitamin C relative to Arjunic Acid).[67]

Arjunic and Arjunolic acid appear to have direct anti-oxidative potential and sequester free radicals; the potency of it appears to be similar to Vitamin C (a few studies suggest more or less, but for the most part is similar)

Edit9. Interactions with Cancer Metabolism

9.1. Genotoxicity/Mutagenicity

Arjuna bark extracts have been found to exert antigenotoxic (protective) properties in response to 4-nitroquinoline-N-oxide,[6] 2-aminofluorene,4-nitro-o-phenylenediamine,[73] and Adriamycin,[70] with the bioactives appearing to be concentrated in the acetone and methanolic extracts.[6][73] Compounds in the ethanolic/acetone extracts appear to protect the DNA from damage induced by mutagens; the bioactives are currently unknown, and this does not appear to occur with the water extract (which is used for cardiovascular health)

9.2. Tumors (Overview)

Oddly, a bacteria that has been noted to produce Taxol (Paclitaxel; chemotherapeutic) has been noted to occur on Arjuna bark;[74][75] this is differnt from the bacteria has been previously found on *Taxus brevifolia* that herb which has a bacterial strain synthesizing Taxol.

Possesses a novel bacteria on the tree which produces the chemotherapeutic Taxol; practical significance unknown, and Arjuna extracts may not have a Taxol content regardless

In response to incubation with an Ehrlich ascites carcinoma (an undifferentiated carcinoma tumor used in research with high differentiation rates and easy transplantation following injections [76]) noted that 9 days of Arjuna *leaf* methanolic extract was able to reduce tumor size (43-67%; nonsignificantly less effective than 20mg/kg 5-fluorouracil) and survival time was extended 43.9% and 87.9% at 100mg/kg and 200mg/kg, with the higher dose not being significantly different than the active control of 5-fluorouracil at 20mg/kg.[9] Arjuna leaf extract appeared to have similar effects on white and red blood cell counts as did 5-fluorouracil.

In vitro, the main bioactive Arjunic Acid at 100mcg concentration appears to induce up to 70% cytotoxicity in these cells and has been noted to influence another cancer cell line (Dalton's lymphoma),[77] with the latter cell line showing up to 90% cytotoxicity with 200mcg/mL of an ethanolic extract of Arjuna Bark[78] and later being confirmed to reduce DLA tumor cell count in a mouse model by 45% and increase lifespan by 60.42% (50mg/kg) and 60% reduce cell count with a 87.50% increase in lifespan (100mg/kg) following oral ingestion of Arjuna bark extract for 10 days, although the higher dose appears to reduce white blood cell count (50mg/kg not affecting WBCs), 2-10mg/kg were also effective on both parameters, but to a lesser degree.[78] One other study using 3-4g/kg Arjuna water extract in mice did not specifically measure tumor size, but in a Dalton's Lymphoma cell line the alterations of anti-oxidant enzymes (Catalase, SOD, Glutatione S-Transferase) effectively normalized the reductions, and reduced LDH levels in serum by 71% (relative to tumor control) at the higher dose.[79]

9.3. Oral Cancer

In a rodent model of DMBA-induced oral carcinogenesis, the water extract of the bark of Arjuna at 500mg/kg appeared to suppress the development of tumors from 100% in control to 30% and reduced average tumor size to 33% of control.[80] This was associated with less adverse histological changes (keratosis, hyperplasia and dysplasia) and improvments in oxidative biomarkers such as TBARS, and the protective effects were thought to be related to anti-oxidant mechanisms.[80]

9.4. Breast Cancer

One study noted that a constituent of Arjuna, the hydrolyzable tannin *Casuarinin*, appears to have anti-proliferative effects in MCF-7 breast cancer cells associated with apoptosis at the G0/G1 phase of cell division.[26]

9.5. Lung

One study has suggested that Casuarinin may induce apoptosis in human non-small cell lung cancer cells (A549) related to apoptosis at G0/G1 from inducing p21/WAF1 via p53.[81]

9.6. Hepatocellular Carcinoma

In isolated HepG2 carcinoma cells, 60-100mg/mL of the ethanolic extract of Arjuna Bark is able to induce an apoptotic morphology unto cells in a concentration dependent manner associated with induction of p53.[82]

In research models of Hepatocellular Carcinoma induced by N-nitrosodiethylamine (one study[83] duplicated in Medline[84]) the ethanolic extract of Arjuna bark at 400mg/kg for 28 days was able to normalize TBARS (indicative of lipid peroxidation) in the liver but not serum of animals bearing N-nitrosodiethylamine tumors; this study did not measure size of tumors *per se*, but suggested this to be a protective effect.[83]

Edit10. Safety and Toxicity

An acute oral toxicity test in rats failed to find any toxicity with up to 2000mg/kg of an ethanolic extract from Arjuna.[46] This lack of harm at the level of 2000mg/kg has been noted elsewhere in rats using bark extracts,[47] although one study using a leaf extract noted that an LD_{50} occurred at 900mg/kg,[9] suggesting higher toxicity associated with leaf based supplements; another Medline entry, which appears to be another publication of the same trial (same authors, sourcing, and introduction) also notes this 900mg/kg LD_{50}.[52]

Scientific Support & Reference Citations

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(Common phrases used by users for this page include terminalia arjuna health benefits, terminalia arjuna calcium content, research+on+benfits+of+arjuna+, bioactivity of terminalia species, as per structure arjunolic acid is aglycone in nature?, arjuna terminalia medicinal uses)

(Users who contributed to this page include Sol, Kurtis Frank)

Appendix C : A Draft Methodology Designed for a laboratory experiment

A laboratory experiment was designed for further analysis to Find the Cadmium absorption property/capacity of *Terminaliaarjuna* seedlings, bark and seed powder.

Aim: Is it possible to use *T. arjuna* Plant to purify water to absorb Cadmium?

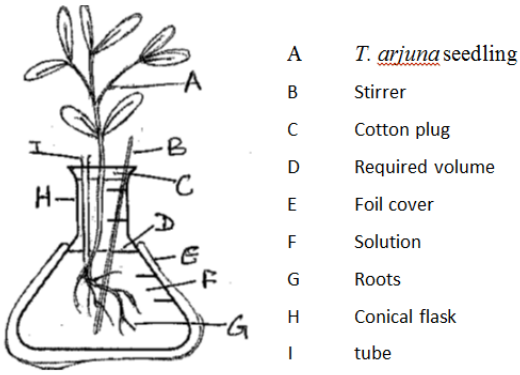


Figure A

Theory:

5.1 Finding the lethal dosage of Cadmium for *T. Arjuna*

Hoagland's Complete Nutrient Solution (25%) is to be prepared (as described inAppendix D)

Prepare series of known concentration of Cadmium and add it to the nutrition solutions. 0, 25, 50, 100, 200,400, 800, 1000 ppm

Introduce *T. arjuna* seedlings to these concentration series,and observe for about one week and find out the maximum concentration(**X**) of Cadmium that will not kill the plant.

Two control solutions

(a) With nutrition solution and Cadmium Xmg/l concentration only without seedling

(b) Distilled water and Cadmium Xmg/l only without seedling

5.2 Finding the Cadmium intake of *T. Arjuna* seedlings

Series (SDL): Known concentration of Cadmium series, **which is less than the lethal dosage** for***T. Arjuna***add to the series of nutrition solutions with known concentration. Place the seedling one each in sealed covered conical flasks (as shown infigure A). Keepthe volume (400 ml) of the solution constant throughout the experiment by adding same nutrition. After carefully stirring the solution, periodically (Once in 3 days) extract samples from the solution and analyse for Cadmium concentration of the sample to identify the Cadmium intake of the seedlings, if any.

Four control solution Series:

(c) With nutrition solution and CadmiumXmg/l concentration only without seedling

(d) Distilled water and CadmiumXmg/l only without seedling

(e) Nutrition solution and seedling only

(f) Distilled water and seedling only

Series of seedlings and solutions

(g) Three replication from 25% Hoagland solution with CadmiumXmg/l with a seedling in each

(h) Three replication from 25% Hoagland solution with CadmiumX-5 mg/l with a seedling in each

(i) Three replication from 25% Hoagland solution with CadmiumX-20 mg/l with a seedling in each

(j) Three replication from 25% Hoagland solution with CadmiumX-50 mg/l with a seedling in each

(14 seedlings)

Determine the Cadmium concentration of these at every 3days or weekly intervals for about 5 weeks, provided plants won't die during this period.

Objective- this will show the absorption of Cadmium by the seedlings with time (if there is any absorption!)

5.3 Finding of Cadmium absorption in *T. Arjuna* seed powder

Series (SD): Add known concentration of Cadmium series to the distilled water. Mix the constant amounts of seed powder (5 mg) to each in sealed covered conical flasks. Keep the constant volume (200ml) of the solution throughout the experiment by adding distilled water. After carefully stirring the solution, periodically (Once in 3 days) extract samples from the solution and analyse the Cadmium concentration of the sample solution to identify the Cadmium intake of the ***T. Arjuna***seed powder.

Control solution Series:

(k) Distilled water and CadmiumXmg/l only (4 for all Cadmium Concentrations)

Series of following solutions mixed with 5mg seedpowder

(l) Three replication of Distilled water with CadmiumXmg/l

(m) Three replication of Distilled water with CadmiumX-5 mg/l

(n) Three replication of Distilled water with CadmiumX-20 mg/l

(o) Three replication of Distilled water with CadmiumX-50 mg/l

(Do we need three replications? If we are adding same amount of seed powder?eg. 5 mg?)

Determine the Cadmium concentration of these series at every 3days or weekly intervals for about 5 weeks.

Objective- this will show absorption of Cadmium by the seed powder with time (if there is any absorption!)

5.4 Finding of Cadmium absorption in *T. Arjuna* bark powder

Series (BK): Add known concentration of Cadmium series to the distilled water. Mix the constant amounts of bark powder (5 mg) to each in sealed covered conical flasks. Keep the constant volume (200 ml) of the solution throughout the experiment by adding distilled water. After carefully stirring the solution, periodically (Once in 3 days) extract samples from the solution and analyse the Cadmium concentration of the sample solution to identify the Cadmium intake of the ***T. Arjuna***bark powder.

Control solution Series:

(p) Distilled water and CadmiumXmg/l only (Do we need 4 for all Cadmium Concentrations?)

Series of following solutions mixed with 5mg bark powder

(q) Three replication of Distilled water with CadmiumXmg/l

(r) Three replication of Distilled water with CadmiumX-5 mg/l

(s) Three replication of Distilled water with CadmiumX-20 mg/l

(t) Three replication of Distilled water with CadmiumX-50 mg/l

(Do we need three replications? If we are adding same amount of seed powder?eg. 5 mg?)

Determine the Cadmium concentration of these series at every 3days or weekly intervals for about 5 weeks

Objective- this will show absorption of Cadmium by the bark powder with time (if there is any absorption !)

Calculations:

a. Volume of Nutrition Solution required

For Experiment 5.1

Volume required per conical flask (X) 400 ml

No. of Flasks requires for 8 Cadmium concentrations with 1control with nutrient = 9 Nos.

Therefore Nutrition Solution requirement for flasks 9 Nos. x 400 ml =**3.6 litres**

For Experiment 5.2

Volume required per conical flask (X) 400 ml

No. of Flasks requires for 4 Cadmium concentrations with 3 seedling replications (Y)

12 Nos.

No. of Control Flask with Nutrient and seedling only 1 No.

No. Control Flask with Nutrient and Cadmium only 4 No.(Or else 1 No.)

Therefore Total No. of 500 ml Conical Flasks 17 Nos.(or else 14)

Therefore Nutrition Solution requirement for flasks 17 Nos. x 400 ml =**6.8 litres**

Nutrition Solution requirement to maintain constant volume? Approx. **3 litres**

Therefore total Nutrition Solution requirement Approx. 15 litres

b. Volume of Distilled water required

For Experiment 5.1 and 5.2

For preparation of Nutrition Solution requirement Approx. 15 litres
For Control Flasks (b,d&f) 400 ml x 3 = 1.2 litres

For Experiment 5.3 and 5.4

Volume required per conical flask (X) 200 ml
No. of Flasks requires for 4 Cadmium concentrations with 3 seed powder replications (Y)
12 Nos.
No. of Control Flask 4 No.
Therefore Total No. of 250 ml Conical Flasks 16 Nos.
Therefore Distilled water requirement for flasks 16 Nos. x 200 ml = 3.2litres
Distilled water requirement to maintain constant volume ? 1litres
Therefore total Distilled water requirement Experiment 5.3 Approx. 5 litres
Therefore total Distilled water requirement **Experiment 5.3 & 5.4 Approx. 10 litres**
Therefore total Distilled water requirement Approx. 26 litres

c. No. of Conical flasks required

For Experiment 5.1 = 10 Nos. 500 ml Conical flasks (Could re-use for Experiment 5.2)
For Experiment 5.2 = 19 Nos. 500 ml Conical flasks
For Experiment 5.3 = 16 Nos. 250 ml Conical flasks
For Experiment 5.4 = 16 Nos. 250 ml Conical flasks
Therefore total *No. of Conical flasks* requirement= *19 Nos. 500 ml Conical flasks and 32 Nos. 250 ml Conical flasks*

d. Plant Material Requirement

For Experiment 5.1 = 8 Seedlings

For Experiment 5.2 = 14 Seedlings

For Experiment 5.3 =60 mg seed powder

For Experiment 5.4 = 60 mg bark powder

Therefore total number of 22 seedlings, 60 mg seed powder and 60 mg bark powder required.

e.*Chemical requirement for preparation of 25% Hoagland Solution (Nutrition Solution) Approx. 15 litres*

		Chemical requirement for preparation of 25% Hoagland Solution[HS] (Nutrition Solution) Approx. 15 litres						
		Chemical	Concentration	Required Volume per liter (in ml)	Required Volume of HS in liter	Therefore Required Volume in ml for 100% solution	Required HS Concentration	Therefore Required Volume in ml
1		$NH_4H_2PO_4$	1 M	1	15	15	25%	3.75
2		KNO_3	1 M	6	15	90	25%	22.5
3		$Ca(NO_3)_2$	1 M	4	15	60	25%	15
4		$MgSO_4$	1 M	2	15	30	25%	7.5
				Required Volume per liter (in gm)*				
5	i.	H_3BO_3		2.86				
	ii.	$MnCl_2.4H_2O$		1.81				
	iii.	$ZnSO_4.7H_2O$		0.22				
	iv.	$CuSO_4.5H_2O$		0.08				
	v.	$H_2MoO_4.H_2O$		0.02				
		* Only 1ml to be added from 5th Mixture per liter						
				(in ml)				
6		Iron Stock (Assaying 85% MoO_3)		0.25	15	3.75	25%	0.9375

f.* $Cd(NO_3)_2.4H_2O$ *Requirement

Preparation of Cadmium concentration solutions

For Experiment 5.1

Volume of the Nutrition Solution per Flask = 400 ml to be decided

Cadmium series of 0, 25, 50, 100, 200,400, 800, 1000 ppm to be prepared

1 mol of Cadmium = 112.411 g

1 mol of $Cd(NO_3)_2.4H_2O$ = 308.48 g

(a) To prepare 1000 ppm Cadmium concentration;

308.48/112.411 X 1g = 2.744g of $Cd(NO_3)_2.4H_2O$ in 1 litre (1000ml)

= 1000 ppm = 1/308.48 X 2.744 M = 0.009 M

Volume of the Solution per Flask = 400 ml

Therefore; Amount of $Cd(NO_3)_2.4H_2O$ to be added;

2.44g/1000mlx 400 ml = **0.976 g$Cd(NO_3)_2.4H_2O$ per flask = 976 mg**

Amount of ***Cadmium*** *added = 112.411g/308.48g X 0.976 g= 0.00324g = 3.24 mg per flask*

(b) To prepare 800 ppm Cadmium concentration;

Volume of the Solution per Flask = 400 ml

Therefore; Amount of $Cd(NO_3)_2.4H_2O$ to be added;

0.976g/1000ppm X 800 ppm = **0.781g per flask = 781 mg**

(c) To prepare 400 ppm Cadmium concentration;

Volume of the Solution per Flask = 400 ml

Therefore; Amount of $Cd(NO_3)_2.4H_2O$ to be added;

0.976g/1000ppm X 400 ppm = **0.390g per flask = 390 mg**

(d) To prepare 200 ppm Cadmium concentration;

Volume of the Solution per Flask = 400 ml

Therefore; Amount of $Cd(NO_3)_2.4H_2O$ to be added;

0.976g/1000ppm X 200 ppm = 0.**195g per flask = 195 mg**

(e) To prepare 100 ppm Cadmium concentration;

Volume of the Solution per Flask = 400 ml

Therefore; Amount of $Cd(NO_3)_2.4H_2O$ to be added;

0.976g/1000ppm X 100 ppm = **0.098g per flask = 97.5 mg**

(f) To prepare 50 ppm Cadmium concentration;

Volume of the Solution per Flask = 400 ml

Therefore; Amount of $Cd(NO_3)_2.4H_2O$ to be added;

0.976g/1000ppm X 50 ppm = **0.049g per flask = 48.8 mg**

(g) To prepare 25 ppm Cadmium concentration;

Volume of the Solution per Flask = 400 ml

Therefore; Amount of $Cd(NO_3)_2.4H_2O$ to be added;

0.976g/1000ppm X 25 ppm = **0.024g per flask = 25 mg**

Therefore; Total Amount of $Cd(NO_3)_2.4H_2O$ for *Experiment 5.1* =

976 mg + 781 mg + 390 mg + 195 mg + 97.5 mg + 48.8 mg + 25 mg

=2,513 mg = 2.51 g

For Experiment 5.2(Lethal concentration to be determined for calculations)

Four Nutrition Solutions (Hoagland's) with different Cadmium concentrations (X mg l^{-1}; X-5 mg l^{-1};X-20 mg l^{-1};X-50 mg l^{-1})

For Experiment 5.3 and 5.4

Four Distilled water solutions with different Cadmium concentrations (X mg l^{-1}; X-5 mg l^{-1};X-20 mg l^{-1};X-50 mg l^{-1})

(Approx - 2.0g x 10 = 20 g ? for *Experiment 5.2, 5.3 and 5.4*)

Equipment and Material Requirement :

a. Total Nutrition Solution requirement Approx. 15 litres

b. Total Distilled water requirement Approx. 26 litres

c. Total *No. of Conical flasks* requirement = *19 Nos. 500 ml Conical flasks and 32 Nos. 250 ml Conical flasks*

d. Total number of 22 seedlings, 60 mg seed powder and 60 mg bark powder required.

e. Chemical requirement for preparation of 25% Hoagland Solution (Nutrition Solution) Approx. 15 litres are as follows;

		Chemical	Concentration	Required Volume
1		NH4H2PO4	1 M	3.75 ml
2		KNO3	1 M	22.5 ml
3		Ca(NO3)2	1 M	15 ml
4		MgSO4	1 M	7.5 ml
5	i.	H3BO3		2.86 g
	ii.	MnCl2.4H2O		1.81 g
	iii.	ZnSO4.7H2O		0.22 g
	iv.	CuSO4.5H2O		0.08 g
	v.	H2MoO4.H2O		**0.02 g**
6		Iron Stock (Assaying 85% MoO3)		0.9375 ml

f.* $Cd(NO_3)_2.4H_2O$ *Requirement

Total Amount of $Cd(NO_3)_2.4H_2O$ for *Experiment 5.1* =2.51 g

For Experiment 5.2, 5.3and 5.4 (Lethal concentration to be determined for calculations)

(Approx - 2.0g x 10 = 20 g ? for *Experiment 5.2, 5.3 and 5.4*)

g. Cotton Wool - To seal the conical flasks

h. Black cartridge paper to cover the Conical Flasks

i. Tube and extraction syringes (do we need 1 each per flask?)

j. string sticks

Procedure:

Exp. 5.1

Sterilized 10 Conical flasks of 500 ml labelled with different concentrations were kept under the room temperature.Added different concentrations of Cadmium to each relevant flask carefully(eg: 25 mg of$Cd(NO_3)_2.4H_2O$ to 25ppm flask). Added the nutrient solution up to 400ml level marked.

8 healthy T. arjuna seedlings taken from Plant Nursery of NavinnaAyurvedic Research Centre and 10 dead culms/ekelscarefully washed of any foreign matters.

Inserted 1 ekel/dead culm in each flask to stir the solution.

Sealed the conical flasks from cotton plugs and covered the flasks with a black cartridge paper.

Observed the plants for about 1 week to determine the approximate maximum concentration which will not be killed the plant (Xmg/lof $Cd(NO_3)_2.4H_2O$)

Exp. 5.2

Four nutrient solutions prepared with different Cadmium concentrations. Another Four solutions of Cadmium concentrations prepared in normal water without the nutrient.

Added 350ml of the nutrient solution for each conical flask with 3 replicates of seedlings for each Cadmium concentrations.

Added 350ml of the solutions of Cadmium concentrations prepared in normal water without the nutrient for each conical flask with 2 replicates of seeds for each Cadmium concentrations.

2 control flask were prepared (a) with Cadmium(Concentration?) with nutrient and (b) with Cadmium with normal water.

Placed a tube in each flask in order to extract the solution for sampling.

Sealed the top of the conical flask with Paraffin Oil and covered the each flask with a foil.

Collection of samples:

X ml of solution was extracted from once in two days from each flask for analysing of Cadmium level(X - Quantity sufficient to analyse Cadmium concentration. Is it necessary to add more solutions?). Added the preservative to the samples, label and stored till analysing for Cadmium concentration.

Preparation of Nutrition Solution:

Hoagland's Complete Nutrient Solution is prepared as per the Recipe of Appendix D

Preservative Method/Solution:

Apparatus:

Measurement of Cadmium level

Measurement of BOD level ?

(From where?Availability of Cadmium testing equipment (Moratuwa/Peradeniya)

Table 1 : Recording of Reading

Cadmium Concentration (per liter)	Seedling (SDL)/ Seed(SD) No.	First week				Second Week				Third Weeek				Fourth Week			
		Mon.	Wed.	Fri.	Sun.	Mon.	Wed.	Fri.	Sun.	Mon.	Wed.	Fri.	Sun.	Mon.	Wed.	Fri.	Sun.
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
5 mg	SDL-5-1-A																
	SDL-5-2-B																
	SDL-5-3-C																
	SD-5-1-D																
	SD-5-2-E																
10 mg	SDL-10-1-F																
	SDL-10-2-G																
	SDL-10-3-H																
	SD-10-1-I																
	SD-10-2-J																
20 mg	SDL-20-1-K																
	SDL-20-2-L																
	SDL-20-3-M																
	SD-20-1-N																
	SD-20-2-O																

Cadmium Concentration (per liter)	Seedling (SDL)/ Seed(SD) No.	First week				Second Week				Third Weeek				Fourth Week			
		Mon.	Wed.	Fri.	Sun.	Mon.	Wed.	Fri.	Sun.	Mon.	Wed.	Fri.	Sun.	Mon.	Wed.	Fri.	Sun.
40 mg	SDL-40-1-P																
	SDL-40-2-Q																
	SDL-40-3-R																
	SD-40-1-S																
	SD-40-2-T																
Control	CL - Nu-U																
	CL - Wa-V																

It is important to do a budgetary allocation of expenses before proceeding with the experiment.

Appendix D : Hoagland Solution Recipe

Recipe for Hoagland's Complete Nutrient Solution

This is made essentially according to the following reference: D.R. Hoagland and D.I. Arnon. The water-culture method of growing plants without soil.Calif.Agr. Expt. Sta. Circ. 347. 1950. There is one change and that is in the form of iron added.

Prepare the following stock solutions (1-6) and use the amounts indicated to prepare 1 liter (final volume) of nutrient solution:

1. 1.00 M $NH_4H_2PO_4$ use 1 ml/l of nutrient solution

2. 1.00 M KNO_3 use 6 ml/l of nutrient solution

3. 1.00 M $Ca(NO_3)_2$ use 4 ml/l of nutrient solution

4. 1.00 M $MgSO_4$ use 2 ml/l of nutrient solution

Micronutrient stocks: combine the following amount of salts in a total volume of one liter of water, and then use 1 ml/ of this entire stock mixture (5) along with the stocks above (1-4) and the iron stock (6) described below to make up a total of 1 litre of nutrient solution.

5. 2.86 gm H_3BO_3

1.81 gm $MnCl_2 .4H_2O$

0.22 gm $ZnSO_4 .7H_2O$

0.08 gm $CuSO_4 . 5H_2O$

0.02 gm $H_2MoO_4 . H_2O$

(Assaying 85% MoO_3)
6. Iron stock: to the above 5 stocks add 0.25 ml of this iron stock for 1 liter of nutrient solution.

To make up the iron stock, take 26.1 g EDTA and dissolve in 286 ml water that has ~19 g KOH. Then dissolve 24.9 $FeSO_4.7H_2O$ in ~ 500 ml water. Slowly add the iron sulfate solution to the potassium EDTA solution and aerate this solution overnight with stirring. The pH rises to about 7.1 and the solution is wine red and very little precipitation occurs. Make to 1 liter final volume and store in a bottle covered with foil (dark).
Note: Hoagland's recipe called for 1 ml of 0.5% iron tartrate stock per liter of nutrient solution but we use the above substitution.

Appendix E : Economic Analysis

1. Background

This appendix provides an assessment of the economic impact of utilizing freely available natural resources for water purification and processing of value added consumable products to introduce income generating methods to vulnerable poor communities in isolated rural areas. The rationale of the project is to improve health condition of vulnerable poor communities by providing safe drinking water and introduce self-sustainable income generating methods which could improve their social and economic standards.

The expected output of the project is as follows:

- Component A: Strengthened research and quality control capacity on applying traditional knowledge on water purification combining with new technological findings
- Component B: Capacity developed in established rural community based organizations on knowledge sharing, application, protection and dissemination
- Component C: Supported in project management and administration
- Component D: Produced value added products from natural resources:
    a. water purification product
    b. beverage/medicine/consumable/any other appropriate product

2. Current Economic Situation in Isolated Vulnerable Poor Communities

The project will be implemented in selected isolated rural areas for vulnerable poor communities.

Majority of these poor communities in Sri Lanka are traditional farmers. They either cultivate paddy or commonly found verities of grain and vegetables under ‘chena’ cultivation. Some of them collect the common vegetable varieties from the forest or

surrounding environment and market those in weekly “Pola” markets in the town areas. Their produces are collect by intermediate vendors for very cheap prices as these communities do not have enough common transport facilities. Some of the villagers work as a construction labourers in town areas, who normally come back to their residence during the weekends, or during the festival seasons. During the day time elderly citizens and mothers with infants are at home. Children travel through long distances to reach schools. The family income of these communities is hardly enough for their daily expenses. Most of these villagers cannot afford at least to travel to hospitals in town areas for medical treatments. The capacities of local hospitals are so poor they prioritized the treatment for patients according to age and influence. Therefore, patients ignore their medical condition till it leads to worse conditions.

3. Project Economic Benefits

The projects aim is to reduce contamination of drinking water sources and maximum utilization of natural resources for adsorption/absorption of chemicals using biological medicinal plants applying traditional/indigenous knowledge on environment combining with finding of new technology.

It is vital that this environmental knowledge among rural communities with its limited resources is utilized efficiently using these methods for a sustainable environment with long term benefits. This environment knowledge could lead to differentiation and specialization of products reducing competition which will lead to the harmony of the society and its health and wellbeing.

There is a very limited amount of sources for safe drinking water in isolated areas where women have to travel long distances to fetch drinking water to their families. Productive time of the nation could be saved, if it is possible to invent a small scale water purification method for safe drinking water for isolated poor rural communities.

In most conventional drinking water supply schemes, at least Chlorine is added for water purification. With the high contamination of water intakes, the utilization of chemicals for water purification levels also rises.

Saving in Chemical cost for Water Treatment

In most of conventional water treatment plants, the basic stages of water purification are aeration, coagulation, flocculation/sedimentation, filtration, disinfection, storage and safe distribution to the consumer.

With the pollution of natural water bodies, the government has to spend enormous amount for water treatment to produce safe drinking water to the nation.

Mainly alum, lime and chlorine are used as chemicals for water treatment. Lime for pH adjustments, alum as coagulant and chlorine for disinfection.

"As an example the total chemical cost for Hiriwadunna water treatment plant is 3.98 US$ / 1000 m^3 of water. The Hiriwadunna water treatment plant is owned and operated by the National Water Supply & Drainage Board (NWSDB) of Sri Lanka. Commissioned by the Government of Sri Lanka in 1963, the plant was rehabilitated in 1997 with financial assistance from Asian Development Bank. The rehabilitation work was conducted between 1993 and 1997, with a total investment cost of US$ 3 million."*(Source: NewTap funded by JWRC, prepared by Lalith Wijesinghe, Chief Engineer, NWSDB/http://www.jwrc-net.or.jp/aswin/en/newtap/report/NewTap_002.pdf , June 2015)*

Following is a table which shows the current urban and rural access to safe water supply in Sri Lanka

URBAN AND RURAL ACCESS TO SAFE WATER SUPPLY, SRI LANKA, 2006–2011

	2006	2007	2008	2009	2010	2011
Population (million)	20.03	20.23	20.43	20.63	20.84	21.05
Pipe-borne coverage (NWSDB &municipalities)	31.10%	32.00%	33.90%	36.50%	38.20%	40.30%
Pipe-borne coverage (other agencies)	1.00%	1.00%	1.00%	1.00%	0.90%	0.80%
Coverage by protected dug wells	34.00%	33.00%	32.00%	31.00%	31.00%	30.00%
Coverage by hand pumps on tube wells	8.00%	8.00%	8.00%	8.00%	8.00%	8.00%
Coverage by rainwater harvesting	2.00%	2.40%	2.70%	3.00%	3.30%	3.60%
Overall access to safe water	76.10%	76.40%	77.60%	79.50%	81.40%	82.60%

Source: NWSDB, MoFP

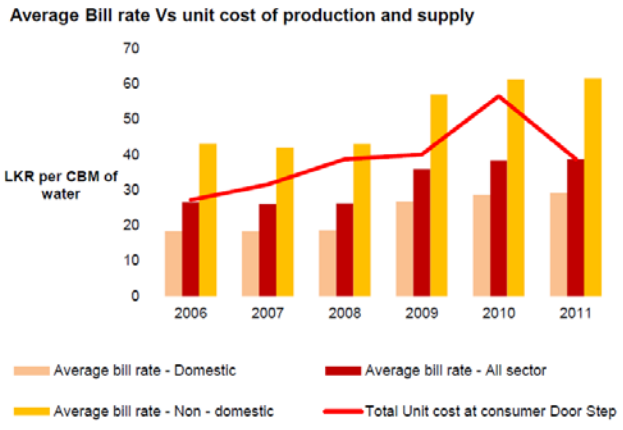
This data clearly depicts that the main way of providing access to safe drinking water is through pipe-bone coverage and protected dug wells.

Following data table shows the capacity and the production of main water supply schemes in Sri Lanka.

Water Treatment Plant	Capacity m^3/day	Production m^3/day
Labugama	59,000	44,300
Kalatuwawa	91,000	83,000
Ambatale	500,000	547,000
Kosgama	2,750	1,100
Penrithwatta	3,000	1,100
Kotabodawatta	3,800	N/A
Kelani Right Bank	180,000	N/A
Raddolugama	N/A	9,000
Pugoda	7,500	7,500
Ranpokunagama	N/A	N/A
Kirindiwela	3,000	750
Kethhena	56,800	N/A
Kandana	60,000	N/A
Ingiriya	450	N/A
Mathugama	200	N/A

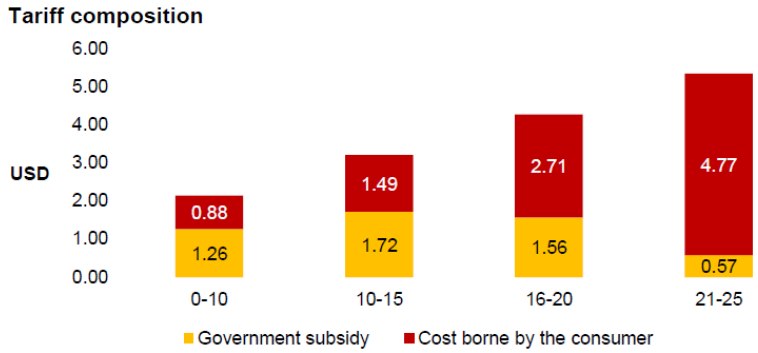
Source: NWSDB

Following data table shows the increase of the Average bill rate for safe drinking water supply.



Source: MoFP Annual report

Following table shows the increase of cost borne by the consumer for water.



Source: MoFP Annual Report

Saving in Loss of Income

Due to unavailability of safe drinking water, inappropriate usage of fertilizers and consumption of unhealthy dietary products, productive life period of communities are shortened.

Savings in Costs to the Health System

" Every year there are 2 million diarrhoeal deaths related to **unsafe water**, sanitation, and hygiene—the vast majority among children under 5. More than one billion people lack access to an improved water source.

Household water treatment and safe storage (HWTS) interventions can lead to dramatic improvements in drinking water quality and reductions in diarrhoeal disease—making an immediate difference to the lives of those who rely on water from polluted rivers, lakes and, in some cases, unsafe wells or piped water supplies." *(Source: World Health Organization Website, Household Water Treatment and Safe Storage. http://www.who.int/household_water/en/, April 2016).*

Economic Benefits from selling Processed Food

Eg. *Terminalia arjuna*

Economic life : More than 60 years

Economics of cultivation / ha No. of plants = 275

Cost of cultivation	:	Rs. 25,000
Maintenance for 15 years	:	Rs. 15,000 to Rs. 25,000

Bark:

Yield dry bark/ha/year after 15 years	:	4 to 4.5 MT
Gross return/ha @ Rs. 50/kg of dry bark	:	Rs. 2000,000 to Rs. 225,000
Net return after 15 years of planting	:	Rs. 160,000 to Rs. 175,000

Yield: From a full-grown tree about 15-20 kg of dry bark is obtained. Production increases upto 25-30 years. About 3 kg fresh bar gives 1 kg on drying.

Medicinal uses:

The bark is of great economic value as it contains 20-24% tannin and used for tanning and dying. It is used in hypertension, and ulcers. The bark is astringent, cooling, anti-dysenteric urinary astringent, anaemia and vitiated conditions of pitta. The bark is popularly used as a cardiac tonic. The bark is useful in diseases of the heart, allays thirst and relieves fatigue. Consumption of barks or bark powder alone will lead to constipation. To avoid this it is to be taken with milk.

(Source: Hand book on Medicinal & Aromatic Plants [NEDFI]from web)

An income generating source:

Currently, other than as timber, the leaves, fruits and plant parts are not commercially used for processing value added products in Sri Lanka. Following photograph witnesses than the timber, after pruning the trees other ***Terminalia arjuna*** plant parts, in a location in North Central Province, are loaded in a truck for disposal.

These leaves, fruits and plant parts could be used for preparation of value added products (eg. Organic fertilizers, beverages, medical and chemical extractions) which will be an income generating method for village communities.

4. Conclusion

Proposed further research on water purification plants, traditional knowledge and finding ways to combine new technology for processing value added products to purify water from natural products and other appropriated products, with involvement of isolated vulnerable communities will improve the economic and social wellbeing of these societies and nation. The finding of innovative methods could be applied as pilot project to find new products to the market which could also improve the health conditions and enhancement of knowledge on nature of the nation.

Appendix F : Project Design and Monitoring Framework

Estimated Project Period (3 Years – from Year 2017 to Year 2019)

1. Impacts of the Project are aligned with:

- Improved health and well-being in selected vulnerable communities in isolated villages
- Increased contribution of the research based indigenous knowledge combined with new technological findings to the economic growth.

2. Outcomes

2.1. Increased consumption of Healthy Drinks and Food Products by Nation

Indicators

By 2021,

a. number of CKD/food poisoning patients reduced in selected vulnerable communities by 20% *(Baseline to be find out from relevant hospitals)*
b. consumption of SLS certified Healthy Drinks in market increased by 20% *(Baseline survey to be carried out)*

Data Sources/Reporting Mechanisms

Central & Local Government Health Inspecting/ MonitoringSystem

Survey Department / Marketing Regulatory Authorities

Independent Surveys

Risks

a. Difficulty in competing with established popular products
b. Resource constrains in the Government
c. Lack of transport and logistic issues

2.2. Increased Safe Drinking Water availability in selected isolated areas with vulnerable communities

Indicators

By 2021,

a. Overall access to safe drinking water increased from 82% to 100% in rural selected communities

Data Sources/Reporting Mechanisms

Annual/Progress reports of NWSDB and Community Development Societies.

Risks

Changes and lack of capacities and facilities of assigned staff, contractors and consultants.

2.3. Increased Income Opportunities in vulnerable communities

Indicators

At least 5,000 members selected from vulnerable communities trained, engaged and received income generated opportunity.

Data Sources/Reporting Mechanisms

Government/Provincial Local Authority reports
Community Development Society Progress reports

Risks

Sustainability of Developed Capacity
Difficulty in selection/retention of long term committed participants

3. Outputs

3.1. Strengthened Research and Quality Control Capacity

Activities

a. *Establish and maintain strong national team for research guidance and activities (Consist of Academic Expertise, University and School Students, Village Representatives including young and elderly citizens with indigenous knowledge)(Q4,2017 – Q4,2019)*
b. *maintain infrastructure/laboratory facilities (Q4,2017 – Q4,2019)*
c. *Conduct Survey (Q4,2017 – Q1,2018)*
d. *EnableLaboratory facilities/Guidance to conduct Research on naturally available products for processing value added products (eg. Water purification / Fertilizer /Food & Beverages)(Q4,2017-Q4,2019)*
e. *Establish Data System for Storage of Research outcome (Q1,2018 – Q4,2019)*
f. *Dissemination of Research outcome, Conduct Training, Development and Awareness Program (Q2, 2018 – Q4,2019)*

g. *Establish supporting legal system for patent, trademark, branding, and permit system for the invented products (Q1,2018)*
h. *Maintain quality controls of products (Q3,2018)*

Indicators

a. *Strong National Research and Quality Control team formed as Consultants (by Q1, 2018)*
b. *Infrastructure and well equipped laboratory facilities allocated from available National Institutions for research activities (by Q1,2018)*
c. *System established for guidance/consultations for Research activities under the project (by Q1,2018)*
d. *Finalized the products formulation to be research under the project (by Q2, 2017)*
e. *Established the Data Management System (by Q1, 2018)*
f. *Conducted Survey and Data Collected on selected natural resources (Q3, 2017)*
g. *Conducted laboratory testing of selected product processed (by, Q1, 2018)*
h. *Established legal system (patent/trademark/branding or permit system for invented products (by Q3, 2018)*
i. *Disseminated Research outcome, Conducted Training, Development and Awareness Program for selected communities (by Q4, 2018)*
j. *90% of invented products in the market are within the quality standards (by Q4, 2018)*

Data Sources/Reporting Mechanisms

a. *National Research and development team quarterly progress reports*
b. *Training workshop course evaluation survey reports*

Risks

To retain the expertise due to limited resources in the country.

3.2. Established and Capacity Developed in Rural Community Based Organizations

Activities

a. *Establish Community Based Organization consist with Vulnerable Rural Communities (Q4,2017)*
b. *Establish a simple data base system, office and product processing setup (Q4,2017)*
c. *Collect the data on available natural resources which are currently not in use and the impact of these resources and coordinate with the Research Unit (Q1,2018)*
d. *Select a Natural Product, Collect it and Process the value added Products with the Guidance of Research Unit (Q2,2018)*
e. *Establish a method of Price control and income generation out of these produce (Q2, 2018)*
f. *Consumption/Market and Distribute these Products under quality control system of Research Unit (Q4,2018)*

Indicators

a. *About 2,000 villagers are involved in collection, processing and value addition of natural products (Q1, 2018)*
b. *About 4,000 families are members of set-up community organization (Q4, 2018)*
c. *At least 4,000 villagers continue to be employed/self-employed and income generated from the knowledge and training they received from the project (Q4, 2019).*

Data Sources/Reporting Mechanisms

a. *Independent monitoring and evaluating specialist reports*
b. *Minutes of the meetings of community organizations*
c. *Quarterly construction reports from engineering firms and provincial local authorities*

Risks

Lack of transport facilities, Cultural practices in rural areas and family commitments could be an obstacle for unemployed community members to group-up and attend training sessions.

3.3. Project Management and Administration Supported

Activities

a. *Appoint PMU and SPCU staff including Project Management Consultant for implementation (Q2, 2017)*
b. *Setup a coordinating, monitoring and implementing system/PPMS (Q3,2017)*
c. *Procure furniture, equipment and vehicle for PMU (Q3, 2017)*
d. *Submit project progress report to Government Control body Monthly (Q2,2017 –Q4,2019)*
e. *Establish the project coordinating committee to review the project progress quarterly basis and submit report to Central Government, Donor and Beneficiaries (Q2, 2017 – Q4, 2019)*
f. *Establish Independent Audit Committees and Annual Audit and Management Review (Q3, 2017 – Q4, 2019)*
g. *Establish a Web-based System to disseminate project news to stakeholders (Q2, 2017)*
h. *Conduct the Project Completion Report (Q1, 2020)*

Indicators

a. *Timely and effective support provided for smooth project implementation*
b. *PMU and SPCUs fully staffed on time*
c. *Quarterly progress/Environment & Social Safeguard/Gender Monitoring reports of satisfactory quality submitted on time*
d. *Project Staff trained on application of best practices of project management in complaint handling, procurement & disbursement, environment and social safeguard and gender*
e. *Timely completion of project activities including Project Completion Report*

Data Sources/Reporting Mechanisms

Quarterly Progress Reports provided by PMU

Risks

a. *Budgetary allocations and Capital Resource constrain within the Country*
b. *Coordination and Finalization of relevant management set up of Executing and Implementing Agencies would be complex*

3.4. Produced value added products from natural resources (eg. Beverage /Water Purification Materials / Organic Fertilizer out of *Terminalia arjuna*)

Activities

a. *Select and Assign specific product and individual responsibilities for Specific Group formed out communities and Research teams (Please refer table **A**for a sample)(Q2,2017)*
b. *Procurement of equipment and materials including laboratory facilities for research (Q4,2017)*
c. *Collection of natural products for research (Q3, 2017)*
d. *Conduct research (Q1,2017 – Q4,2018)*
e. *Finalize the Value added Product/ preparatory method (Q1,2019)*
f. *Community Trained on preparation and utilization of product (Q2, 2019)*
g. *Community market/utilize the product (Q3, 2019)*

Indicators

a. *At least 500 freely available natural products are processed as value added products*

Data Sources/Reporting Mechanisms

a. *Independent monitoring and evaluating specialist reports*
b. *Minutes of the meetings of community organizations*
c. *Project Management Unit Quarterly Progress Report*
d. *Government / Provincial Local Authority reports*

Risks

a. *Differences in preferences of selected products by the communities*
b. *Team agreement for selection of variety of product*
c. *Balancing environment without over usage of a particular resources*

Inputs

Government

Private Sector Group 1

Private Sector Group 2

Donor Agency Group 1

Donor Agency Group 1

Community

Table A : Assignment of Work for Selected Group

	Group A - Beverages			***Group B - Medicine***			***Group C - Water Purification Product***		
Out of Terminalia arjuna	***Assigned work***	***Specific Members***	***Budget In LKR***	***Assigned work***	***Specific Members***	***Budget In LKR***	***Assigned work***	***Specific Members***	***Budget In LKR***
Members from related Government Institute					*Ministry of Health /Ministry of Indigenous Medicine*			*MoWSCT/NWSDB*	
Members of Research Team	*(a) Provide knowledge and guidance* *(b)Design research methodology* *(c)Support and Quality control*						*Chemical Engineering*		
Members from University	*(a) Support/Conduct for research and initial processing* *(b)Training communities with best practices for processing and application*								
Members from School Children	*(a) Support for collection* *(b) Support/Conduct research* *(c) Support for data base* *(d) Support for processing*								
Members of Community	*(a) Knowledge of Traditional/Possible Beverages* *(b) Knowledge of Available sites* *(c) Collection of materials* *(d) Support for processing* *(e) Support for Marketing/employment*								