Increased-rate stability studies for St John’s wort 
(*Hypericum perforatum*), *Ginkgo biloba* and Kava Kava 
(*Piper methysticum*) under unfavourable environmental 
conditions.

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PREFACE

I hereby confirm that this is my own work, and that it has not been submitted to any other institution.

Andre Marais
I would like to extend a warm word of thanks to the following:

- Dr Kobus Eloff without whose support and wisdom I would not have been able to complete this project.

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- Tanya for her patience and understanding.

- My Creator for His abundant blessings.
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SUMMARY

This was a chemical laboratory study. The main focus was to evaluate the chemical stability of Hypericum perforatum (St John’s wort), Ginkgo biloba and Piper methysticum (Kava Kava) under unfavourable environmental conditions. Different dosage forms representing the same amount of active ingredients for each were used. Some of the dosage forms were self manufactured according to Good Manufacturing Practice. Samples of the dried powder of each plant was also exposed to a series of gamma-radiation.

Acetone was used as an extractant for all three plants, after evaluating and discarding the extraction method stipulated in the British Herbal Pharmacopoeia. Identification of the different plants were carried out by means of Thin Layer Chromatography. The in-house developed mobile phases EMW, BEA and CEF, showed better separation and visibility compared to the mobile phases used in the British Herbal Pharmacopoeia. The plates were sprayed with either vanillin or p-anisaldehyde for optimal visualization of the separated compounds.

After the specified period of 6-months, comparative TLC was performed on all samples. This was achieved for each plant by applying all samples stored at a specific condition i.e. 25°C, on the same plate. The samples were stored at low temperature after exposure to the specific time interval.

Quantitative analysis was performed by spectrophotometry, and high pressure liquid chromatography. The data obtained from these analytical methods, were used to evaluate the relative chemical stability of each dosage form. The relationship between the quantitative data and the qualitative changes in the TLC fingerprints, were compared, hoping to achieve a common pattern relating to the stability.

The order of the reaction as well as the reaction rate constant (k) for each dosage form was calculated, except for kava kava. The shelf-life (t_{90}) was calculated using the analyzed data obtained by spectrophotometry or HPLC. The relevance of conventional pharmaceutical calculations in the prediction of shelf-life, by means of accelerated stability tests, was investigated for the possible application to herbal products.
The effects of gamma radiation on the degradation of the chemical compounds present in each plant, was evaluated.

After an evaluation of all the relevant data, it seemed that the tablet-dosage forms were equally effective regarding stability, compared to the capsules. Liquid extracts appeared to be less stable than the extract capsules. The extract capsules seemed to degrade more rapidly than the herbal tablets or herbal capsules. Exposure to low dose radiation (4.4 kGy) did not seem to have an influence on the stability. It was evident that some herbs were more sensitive to sunlight or heat than others.

In general, all three of the chosen plants seemed to be relatively stable if stored in the specified conditions. It seemed valid for the shelf-life to be expressed as two years.
Die hoof klem van hierdie projek was om die chemiese stabiliteit van drie van die mees algemeen gebruikte natuurtlike medisyne in Suid-Afrika te ondersoek. Verskillende doseervorme van St John’s wort (*Hypericum perforatum*), *Ginkgo biloba*, en Kava Kava (*Piper methysticum*), is vir die ondersoek gebruik. Uitsluitend Kava Kava, het die verschillende doseervorme elkeen oor dieselfde hoeveelheid aktiewe plant materiaal beskik. Waar gesikte doseervorme nie beskikbaar was nie, is dit self vervaardig. Alle vervaardiging het geskied onder sogenaamde Goeie Vervaardigings Praktyk (GMP), ’n vereiste gestel deur die medisyne-beheer-raad tydens vervaardiging van alle etiese produkte. Monsters van die gedroogde poeier van elke plant is ook blootgestel aan verschillende dosisse van gamma-bestraling.

In al drie plante was asetoon die gekose ekstraheermiddel, nadat daar besluit is om nie die ekstraksie metode, soos verwat in die British Herbal Pharmacopoeia, te implementeer nie. Identifikasie van die verschillende plante is ook uitgevoer deur middel van dunlaag chromatografie. Met die gebruik van ons eie ontwikkelde mobiele fases, EMW; CEF en BEA in die plek van die mobiele fases soos vermeld in die BHP. Die skieding en visualisering van die bande in die verschillende plante was meer duidelik waarnembaar met ons eie metodes. Die verschillende dunlaagplate is gesproei met vanillien of anysaldoeid en die optimale visualisering van die geskeide komponente.

Na die verstryk van die gespesifiseerde 6-maande, is die verschillende dunlaagplate met mekaar vergelyk. ’n Goeie vergelyking kon getref word deur elke monster wat by dieselfde kondisie onderworpe was, op dieselfde plaat aan te wend bv. Al die monsters van kava kava wat by 25°C gestoor was, is op dieselfde plaat aangewend. Na die onttrekking van die monsters by elke gekose tydsinterval, is it by ’n lae temperatuur gestoor totdat analyses daarop gedoen kon word.

Kwantitatiewe analyse is uitgevoer deur gebruik te maak van spektrofotometrie sowel as hoë-drup vloeistof chromatografie. Die data wat deur hierdie analitiese metodes verkry was, is gebruik om die chemiese stabiliteit in elke doseerform te evalueer. Die verwantskap tussen die ge-analiseerde data en die dunlaag-identifikasie profiele is ondersoek, met die hoop dat daar ’n sekere mate van ooreenstemming getoon kon word, of ’n waarneembare patroon wat ’n moontlike toepassing op die stabiliteit kon hê.
Die orde van die chemiese afbraak, sowel as die reaksie snelheids-konstante \( (k) \) is ook vir elke produk, behalwe Kava Kava bepaal. Die rakleeftyd \( (t_{90}) \) is ook vir elke produk bepaal deur die waardes uit die analises verkry uit spektrofotometrie en hoë-druk vloeistof chromatografie te gebruik. Die toepaslikheid van konvensionele farmaseutiese vergelykings in die skatting van 'n rakleeftyd, deur gebruik te maak van versnelde stabilitietstoetse, is ook ondersoek. Dit is uitgevoer met die hoop van 'n moonlike toepassing in natuurlike medisyne.

Na evalueringe van al die relevante data het dit gebleek dat die tablette net so effektief, betreffende die stabiliët, is in vergelyking met die kapsules. Verder het dit ook gebleek dat die vloeistof ekstrakte minder stabiel was as die ekstrak kapsules. Die ekstrak kapsules toon 'n vinniger afbraak as die tablette of die fyn-krui kapsules. Blootstelling aan 'n lae dosis bestraling (4,4 kGy) het geen noemenswaardige invloed op die stabiliteit getoon nie.

Dit was duidelik dat sekere produkte meer sensitief teenoor blootstelling aan sonlig en hoë temperature was, as ander. Oor die algemeen het dit gebleek dat al drie hierdie plantes oor 'n aanvaarbare stabiliët beskik, tensy dit onder die regte bewaringstoestande gestoor word. 'n Vervaldatum van twee jaar op hierdie produkte blyk aanvaarbaar te wees.
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<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>BEA</td>
<td>Benzene/Ethanol/Ammonium hydroxide [18/2/0.2 v/v/v]</td>
</tr>
<tr>
<td>BHP</td>
<td>British Herbal Pharmacopoeia</td>
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<tr>
<td>BP</td>
<td>British Pharmacopoeia</td>
</tr>
<tr>
<td>CEF</td>
<td>Chloroform/ethyl acetate/formic acid [10/8/2 v/v/v]</td>
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<tr>
<td>DI water</td>
<td>De-Ionized water</td>
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<tr>
<td>EC</td>
<td>Extract capsule</td>
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<tr>
<td>EMW</td>
<td>Ethylacetate/methanol/water [10/1/35 v/v/v]</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma Amino Butyric Acid</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practice</td>
</tr>
<tr>
<td>G-protein</td>
<td>Gluco-protein</td>
</tr>
<tr>
<td>HC</td>
<td>Herbal capsule</td>
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<tr>
<td>HIV</td>
<td>Human Immune deficiency Virus</td>
</tr>
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<td>HPLC</td>
<td>High Pressure Liquid Chromatography</td>
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<td>HT</td>
<td>Herbal Tablet</td>
</tr>
<tr>
<td>LIQ</td>
<td>Liquid Extract</td>
</tr>
<tr>
<td>MCC</td>
<td>Medicine Control Council</td>
</tr>
<tr>
<td>MAO</td>
<td>Monoamine oxidase</td>
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<tr>
<td>MeOH</td>
<td>Methanol</td>
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<td>OTC</td>
<td>Over the Counter</td>
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<tr>
<td>PAF</td>
<td>Platelet activation factor</td>
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