MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

INSTITUTE OF TRADITIONAL MEDICINE

RESEARCH REPORT

(Sida-MUHAS FUNDING)

TITLE:

PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON FOUR THREATENED *GARCINIA* SPECIES OF TANZANIAN ORIGIN

PRINCIPAL INVESTIGATOR:

JOSEPH JANGU MAGADULA

Dip (Ed), B.Sc (Ed), M.Sc (Chem), PhD

Email: jmagadula@gmail.com

Phone: +255 754 568702

1. INTRODUCTION

This report describe the research activities done for the proposal titled "PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON FOUR THREATENED *GARCINIA* SPECIES OF TANZANIAN ORIGIN" funded by Sida-MUHAS through the Directorate of Research and Publications(DRP).

2. RESEARCH ACTIVITIES

The major research activities involved **collection of plant materials, biological testing** and **isolation of active ingredients**

During the course of the research period the following activities were done:

(i) Plant sample collection

The stem barks of each plant were collected from signified places as indicated in Table below:

	Species	Location in Tanzania	Status
1	*Garcinia acutifolia*	Litipo forest-**Lindi**	Endemic
2	*G. indica*	Migombani and Mtoni areas-**Zanzibar**	Exotic
3	*G. pachyclada*	Ngolotwa (Kasanga area)-**Rukwa**	Indigenous
4	*G. smeathmannii*	Mukugwe river (Kibondo)-**Kigoma** .Mwalesi River Rungwe)-**Mbeya** .Upper Ruhudje river and Lupembe area-north of the river-Njombe-**Iringa**	Indigenous

(ii) Sample preparations

Plant materials were chopped, dried and grinded into powder form. Plant materials were soaked sequentially in solvents of increasing polarities for 48 hrs while the solvent were removed by concentrating on a rotary evaporator at reduced pressure to obtain the crude extracts.

(ii) Bioassaay

The crude extracts of the stem barks of the plants under this investigation were screened for toxicity in BST, anti-HIV-Protease inhibition and antioxidant

(a) Table 1: BST results for extracts from 4 *Garcinia* species

Extract	LC_{50} (µg/ml)	95% CI (µg/ml)
GASE	289.12	267.12 - 321.20
GISE	89.23	76.72 - 113.24
GPSE	427.85	356.90 - 754.23
GSSE	236.40	120.34 - 653.10
Cyclophosphamide*	16.3	10.6 - 25.1

* = positive standard; GASE = *Garcinia acutifolia,* stem bark soaked in ethanol; GISE = *Garcinia indica,* stem bark soaked in ethanol; GPSE = *Garcinia pacchyclada,* , stem bark soaked; GSSE = *Garcinia smeathmannii,* stem bark soaked in ethanol

(b) Table 2: DPPH antioxidant activities of crude extract s from 4 *Garcinia* species

Extract	*Antioxidant, DPPH[a] (µmol TE/g)
GASE	2,123 ± 150
GISE	2,877 ± 215
GPSE	1,876 ± 107
GSSE	2,403 ± 271
Chlorogenic acid (Standard)	3,056 ± 157

* Antioxidant activities were expressed as means ± SD of three determinations, a = 1,1-Diphenyl-2-picrylhydrazyl;

(c) Table 3: Anti-HIV-1 protease activity of crude extracts of 4 *Garcinia* species

Extract	IC_{50} (µg/ml)*
GASE	37.9
GISE	13.2
GPSE	46.4

GSSE	24.1
Acetyl pepstatin (+ve control)	2.2

* Anti-HIV-1 Protease activities were expressed as means ± SD of three determinations

(iv) Isolation of pure compounds

The isolation of compounds from each crude extract was done using chromatographic techniques using a series of organic solvents of different polarities. Pure samples were obtained as indicated below.

(a) From *Garcinia acutifolia* (Stem bark)

Chromatographic analyses of the crude extract lead to the isolation of two pure samples, GASE 23.6 (**26.0 mg**) and GASE 32.1 (**10.5 mg**).

(b) From *Garcinia indica* (Stem bark)

Chromatographic analyses of the crude extract of *G. indica* lead to the isolation of four pure samples, GISE 14.0 (**16.7 mg**), GISE 25.1 (**20.6 mg**), GISE 37.0 (**6.5 mg**) and GISE 42.4 (**11.6 mg**).

(c) From *Garcinia pachyclada* (Stem bark)

Chromatographic analyses of the crude extract of *G. pachyclada* gave two pure samples, GPSE 8.0 (**12.0 mg**) and GPSE 22.5 (**8.6 mg**)

(d) From *Garcinia smeathmannii* (Stem bark)

Isolation of compounds from the crude extract of *G. smeathmannii* using chromatographic techniques gave three pure samples, GSSE 4.8 (**10.0 mg**), GSSE 12.2 (**8.6 mg**) and GSSE 36.8 (**7.9 mg**)

(v) Spectroscopic analyses

(a) NMR analyses

Isolated pure samples were sent to France for spectroscopic experiments. ^{1}H- and ^{13}C-NMR, COSY, HMQC, HMBC and NOESY spectra were recorded on a Bruker Avance DRX-500 (500MHz) NMR spectrometer. Chemical shifts are expressed relative to the deuterated solvent (MeOH-d4 : δH/δC, 3.31/49.01 ppm).

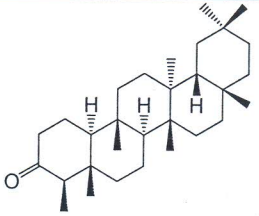
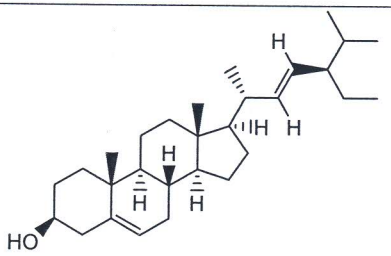
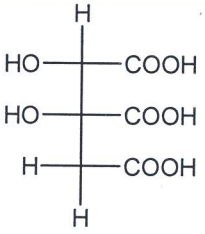
(b) MS analyses

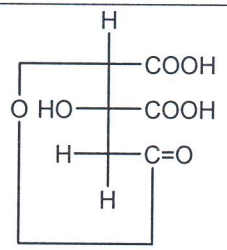
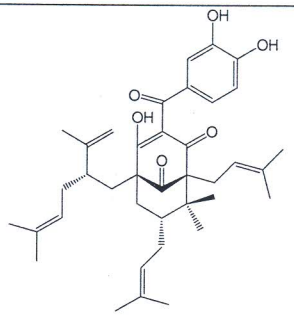
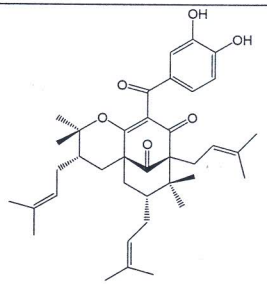
Mass analyses were performed using a Bruker ESI-TRAP Esquire 3000 Plus spectrometer.

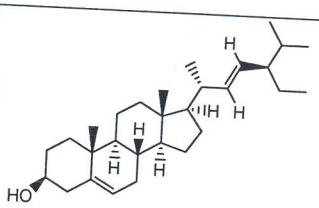
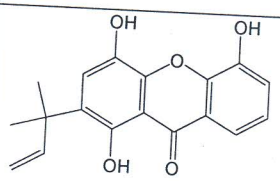
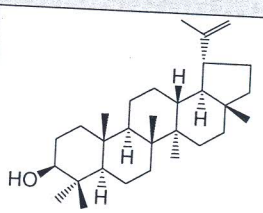
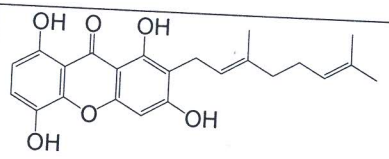
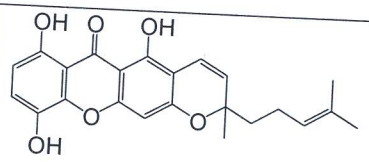
(vi). Identification of Isolated compounds

After getting the spectra from our collaborator in France, we engaged in structure elucidation that lead to identification of all compounds as indicated in Table 4.

Table 4: Compounds isolated from 4 *Garcinia* species

Plant	Compound code	Name	Structure	Reference
G. acutifolia	GASE 23.6	Friedelin		Klass and Tinto, 1992
	GASE 32.1	Stigmasterol		
G. indica	GISE 14.0	(−)-Hydroxycitric acid,		Jayaprakasha and Sakariah, 2002

	GISE 25.1	Hydroxycitric acid lactone		Jayaprakasha and Sakariah, 2002
	GISE 37.0	Garcinol		Kaur et al., 2012
	GISE 42.	Isogarcinol		Kaur et al., 2012

G. pachyclada	GPSE 8.0	Stigmasterol		
	GPSE 22.5	l2b-hydroxy-des-D-garcigerrin A		Sordat-Diserens *et al.*, 1989
G. smeathmannii	GSSE 4.8	Lupeol		Siddqui et al., 1988
	GSSE 12.2	Smeathxanthone A		Komguem, et al., 2005
	GSSE 36.8	Smeathxanthone B		Komguem, et al., 2005

(v) Biological testing of pure compounds

The bioassay of the pure compounds was done only for compounds with reasonable amounts. Hence, compounds **1**, **2**, **3** and **4** showed lower activity as compared to the crude extracts (Table 2).

Compound	LC_{50} (µg/ml)	DPPH (µmol TE/g)	IC_{50} (µg/ml)
Friedelin	462.48	568 ± 120	> 100
Stigmasterol	305.34	239 ± 671	> 100
(−)-Hydroxycitric acid	632.10	2,346 ± 980	> 100
(−)-Hydroxycitric acid lactone	438.47	2,156 ± 521	> 100
Garcinol	36.90	2,254 ± 734	54.7
Isogarcinol	45.21	2,068 ± 142	76.9
l2b-hydroxy-des-D-garcigerrin A	367.20	1,478 ± 847	36.2
Lupeol	298.31	752 ± 190	> 100
Smeathxanthone A	187.19	1984 ± 165	51.2
Smeathxanthone B	283.10	2,215 ± 120	63,8
Cyclophosphamide (standard)	16.3		
Chlorogenic acid (Standard)		3,056 ± 157	
Acetyl pepstatin (+ve control)			2.2

3. DISSEMINATION OF RESULTS

(a) Publication

One manuscript is under preparation to be submitted to the peer reviewed journals

(b) Presentation to the conference

Part of the results were presented to the NAPRECA Symposium in Khartoum, December 2013 under the title "Phytochemical and Pharmacological Studies of some Tanzanian Medicinal Plants

4. FUNDS

The funds were disbursed as requested, used according to the planned activities and retired on time

5. CONCLUSION AND RECOMMENDATION

The objectives of this study were achieved to about 99%. During this study, it was observed that, the disbursed funding was not enough to cover all the planned activities. Hence it is hereby recommended that funds should be increased as inflation may be the reason. Furthermore, semi-synthesis and/or total synthesis has to be planned for pure isolated compounds.

6. REFERENCES

Jayaprakasha, G.K., Sakariah, K.K. Determination of Organic Acids in Leaves and Rinds of *Garcinia indica* (Desr.) by LC. *Journal of Pharmaceutical and Biomedical Analysis*, **2002**, 28 379–384

Kaur, R., Chattopadhyay, S.K., Tandon, S. Sharma, S. Large Scale Extraction of the Fruits of *Garcinia indica* for the Isolation of New and known Polyisoprenylated Benzophenone Derivatives, *Industrial Crops and Products*, **2012**, 37, 420-426

Klass, J., Tinto, W.F. Friedelane Triterpenoids from *Peritassa compta*: Complete ^{1}H and ^{13}C assignments by 2D NMR spectroscopy. *Journal of Natural Products, 1992*, 55, 1626-1630

Komguem, J., Meli, A.L., Manfouo, R.N., Lontsi, D., Ngounou, F.N., Kuete, V., Kamdem, H.W., Tane, P., Ngadjui, B.T., Sondengam, B.L., Connolly, J.D. Xanthones from Garcinia

smeathmannii (Oliver) and their antimicrobial activity, *Phytochemistry*, **2005**, 66, 1713-1717

Siddqui, S., Hafeez, F., Begum, S., Siddiqui, B.S. Oleanderol; A new pentacyclic triterpenes from the Leaves of *Nerium oleander*. *Journal of natural products*, **1988**, 51, 229-233.

Sordat-Diserens I, Marston A, Hamburger M, Rogers C, Hostettmann K. Novel Prenylated Xanthones from *Garcinia gerrardii* HARVEY. *Helv. Chim. Acta*, **1989**, 72, 1001-1007

.....
Dr. Joseph J. Magadula

.....