

Protocol Title: A cross-over study comparing the bioavailability Phytofare^(R) against Phytofare^(R)Pheroid[®] Catechin Complex and a generic green tea extract

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Study Design: Cross-over blind for the participants and laboratory

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Note: The report is accurate reflection of the results of this study. Further analysis of the results are required.

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LIST OF ABBREVIATIONS AND SYMBOLS

AE	adverse event
ALT	alanine transaminase
AST	aspartate aminotransferase
BMI	body mass index
BUN	blood urea nitrogen
°C	degree Celsius
CBC	complete blood count
Cl	chloride
cm	centimetre
EC	epicatechin
ECG	epicatechin-3-gallate
ECGC	epigallocatechin-3-gallate
EGC	epigallocatechin
EDTA	diaminoethanetetraacetic acid
e.g.	for example
<i>et al</i>	and others
g	gram
GCP	Good Clinical Practice
GGT	GAMMA-GLUTAMYL TRANSFERASE
i.e.	that means
lbs	pounds
ICH	International Conference of Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
K	potassium
kg	kilogram
L	liter
m	meter
mg	milligram
ml	milliliter
Na	sodium
RBC	red blood cells
SAE	serious adverse event
SOP	standard operating procedure
SST	serum separating tube
TPD	Therapeutic Products Directorate
ULN	upper limit of normal
WBC	white blood cell

1. INTRODUCTION

Flavan-3-ols, are categorized based on their degree of polymerization, oxidative state, and the substitution pattern of the B and C rings (Beecher, 2003). Five major flavan-3-ols referred to as catechins are commonly found in the diet: catechin (C), Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) are major catechins present in green tea, (McKay & Blumberg, 2002). In green tea, 59% of the catechins are EGCG, 19% are EGC, 13.6% are ECG and 6.4% are EC (McKay & Blumberg, 2002).

The bioavailability of tea flavanols has received attention in many human clinical studies (Reddy, *et al.* 2005; Kyle, *et al.* 2007; Chow *et al.* 2003; Henning, *et al.* 2003). Flavanol-3-ols are rapidly absorbed from green and black tea products following consumption, with plasma C_{max} levels being reached between 0.5 and 2h post administration and baseline levels being established within 8 to 12 hours post administration (Neilson & Ferruzzi, 2011). The bioavailability of EGCG and ECG are reported to be lower than EGC and EC however EGC and EC are the most abundant flavan-3-ols in humans (Henning, *et al.* 2003; Stalmach, *et al.* 2009; Stalmach, *et al.* 2010). Another study also reported that after ingestion of green tea, EGCG, EGC, and EC concentrations peaked in plasma at 1 to 2 hours and gradually reduced to undetectable levels after 24 hours (Lee, *et al.* 2002).

Absorption of flavan-3-ols is thought to be a complex multi-step process and is influenced by the botanical source of and the profile of the flavan-3-ol, the type and extent of the processing and the formulation and composition of the product (Neilson & Ferruzzi, 2011). In order for efficient bio-accessibility, flavan-3-ols must be released from molecular associations with other food components and the food matrix, be soluble in the layer of water that protects the surface of the intestinal enterocytes surface and be stable under gastrointestinal conditions (Neilson & Ferruzzi, 2011). An oral administration of tea catechins to rats has indicated that tea catechins are absorbed intestinally (Okushio, *et al.* 1996). Auger *et al.* (2008) reported the ileal recovery rates were 12%-36% for EC, 47%-59% for EGCG, 53-74% for GCG and 26-34% ECG in participants with an ileostomy consuming a green tea extract (Auger, *et al.* 2008). Although this study was in subjects with no colon, the results are comparable to those reported in *in vitro* studies.

Plandai's proprietary hydrodynamic sheering process releases the phytonutrients from their sources and alters the stereochemical structure by changing the trans-isomers' into their cis forms and/or changing the cis-isomers into the trans form to accomplish a 50:50 ratio of cis:trans isomer which can be readily absorbed by human tissue. By creating a desired cis:trans isomers ratio, Plandai's Phyofare™ green tea Gallate Catechin extract may deliver a highly bioavailable antioxidant-rich material at greater levels than other green tea extracts.

2. STUDY OBJECTIVE

The objectives of this study were twofold:

- (i) to compare the bioavailability of Phytofare^(R) against a generic green tea extract. and to analyse the catechin profiles of trial participants in terms of (i) bioavailability and (ii) stability (Arms 1 and 2).
- (ii) to investigate whether the formulation of Phytofare^(R) in Pheroid[®] increases the catechin content of the plasma of trial participants or the in-body stability of the catechins.

The specific aim of the study was to evaluate the comparative bioavailability and stability of epigallocatechin, epicatechin, epigallocatechin gallate, epicatechin gallate, catechin gallate, gallic acid and total catechin.

3. STUDY DESIGN & POPULATION

This study is a single-center, non-randomized but blind crossover, 24-hour bioavailability study with two arms. The details of the study was presented in a previous report. Adverse events were assessed at each study visit as well as on the first day on taking the capsules. Analytical methods were developed and are fully described in a previous report. Liver function of the subjects was monitored at the screening phase and after each arm of the study. This study was conducted with the highest respect for the individual participants (i.e., participants. The outputs of the study are the concentration-time curves (AUC) for plasma epigallocatechin, epicatechin, epigallocatechin gallate, epicatechin gallate, catechin gallate, gallic acid and total catechin which will be determined by LC-MS-MS. Additional endpoints include time at maximum concentration (T_{max}) and maximum concentration (C_{max}) for plasma epigallocatechin, catechin epicatechin, epigallocatechin gallate, epicatechin gallate, catechin gallate, gallic acid and total catechin.

4. INVESTIGATIONAL PRODUCT

The investigational product raw material was provided by the sponsor. The investigational raw material, formulations and resultant capsules were carefully stored at the study site in a lockable, limited access area, accessible only to study team personnel in compliance with pertinent regulations. The three investigational products were:

Arm 1: a reference (commercial) product (2x200mg per day)

Arm 2: the phytofare^(R) test product (2x200mg per day)

Arm 3: the phytofare^(R)-pheroid[®] (ph²) test product (2x200mg per day)

The dosage form for all arms was in liquid capsules, which allowed for blinding of the study. The products were stored at room temperature and were not exposed to direct sunlight or heat.

The investigational products were manufactured by the DST/NWU Preclinical Drug Development Platform, North-West University, Potchefstroom Campus, South Africa.

The washout period between the three arms was planned to be 14 days but was in fact much longer due to supply issues.

5. RESULTS

Data required for the analysis were acquired from source documentation (including laboratory reports) and entered into a database. All data points entered into the study database are source data verified.

Bioavailability parameters, including the area under the curve ($AUC_{(0-24h)}$), the maximum observed concentration (C_{max}) and time of maximum concentration (T_{max}) for the three study formulations were calculated. Descriptive statistics, including means and standard deviations were calculated for each investigational product. Repeated measures analysis of variance will be used to compare the products with respect to these endpoints. Probability values less than 0.05 are considered to be statistically significant. Effect sizes will be calculated. The statistical analysis performed used industry recognized statistical software (SAS or SPSS). The calibration curves and catechin profiles for the investigational products were presented previously and are available upon requests. The comparative catechin averages detected for all participants at each of the time points of all arms are given in table 1 below.

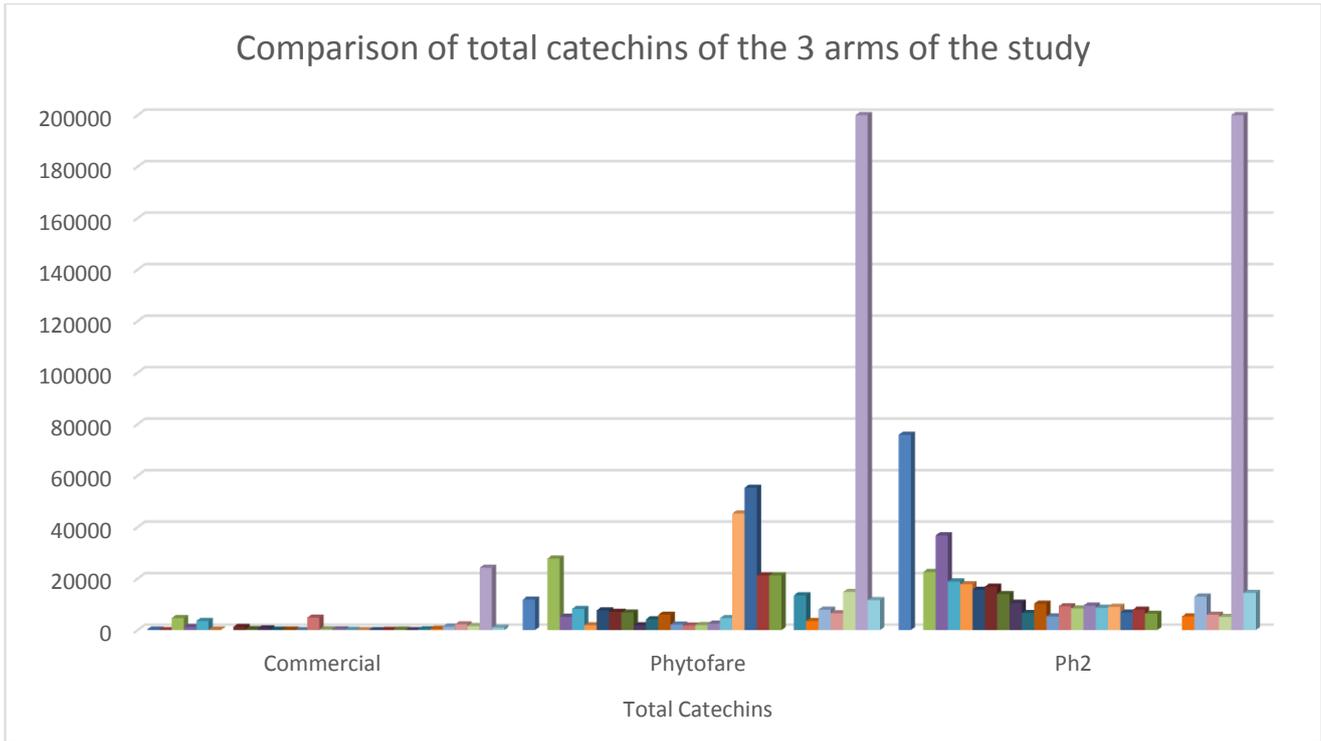
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No	Total ARM 1								
	Epigallocatechin	Gallocatechin Gallate	Epicatechin	Epicatechin gallate	Gallocatechin	Epigallocatechin Gallate	Catechin	Catechin Gallate	Total Catechins
4	18	0	5	0	1	155	3	0	183
5	0	0	15	0	0	61	0	0	76
6	591	527	187	255	192	2882	3	0	4636
7	53	101	154	201	49	612	19	0	1189
8	408	362	48	114	118	2443	13	5	3511
9	0	0	0	0	0	133	0	0	133
11	77	151	0	0	66	725	190	0	1209
12	74	0	1	3	12	276	3	0	369
13	31	17	6	0	72	503	0	0	629
15	0	0	4	0	7	213	0	0	224
16	0	6	20	0	122	13	0	21	182
17	0	0	0	0	0	0	0	0	0
18	594	834	196	81	322	2715	67	0	4808
19	0	0	18	0	18	241	0	0	276
21	52	0	33	0	8	214	0	0	307
22	0	0	0	0	11	52	18	8	89
23	0	0	0	0	2	0	0	0	2
24	0	0	0	0	1	0	0	0	1
25	0	0	0	0	0	95	0	0	95
26	0	0	0	0	2	170	0	0	172
27	0	0	0	0	0	90	0	0	90
28	7	0	0	0	12	286	2	0	306
29	0	13	0	0	0	480	0	0	493
30	28	58	57	56	149	977	10	9	1344
31	199	123	170	141	155	1281	140	35	2245
32	52	78	40	10	43	1306	59	31	1619
Total ng/ml	2184	2271	953	861	1362	15921	525	109	24186
Average ng/ml	84	87	37	33	52	612	20	4	930

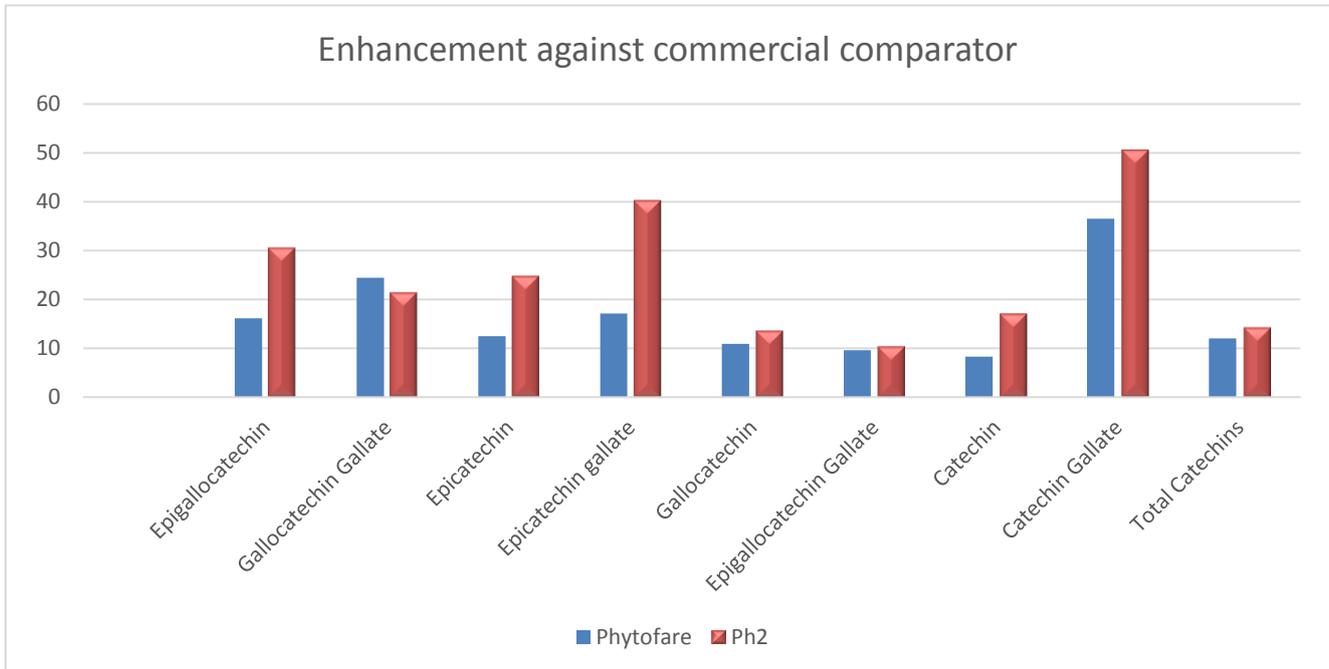
Total ARM 2									
No	Epigallocatechin	Gallocatechin Gallate	Epicatechin	Epicatechin gallate	Gallocatechin	Epigallocatechin Gallate	Catechin	Catechin Gallate	Total Catechins
4	1400	1846	467	533	581	6704	189	145	11865
6	3617	4552	965	1407	1420	15223	346	220	27750
7	640	867	292	153	274	2720	101	125	5173
8	1169	1338	464	423	617	3963	177	70	8222
9	47	401	0	93	19	1217	8	77	1862
10	1267	1296	191	549	372	3823	110	48	7656
11	1266	1108	474	255	599	3107	83	183	7074
12	1039	976	605	388	512	2944	235	66	6766
13	52	402	0	48	46	1255	68	14	1884
15	457	650	63	235	172	2578	36	28	4220
16	344	1087	154	265	194	3781	43	64	5933
17	250	425	42	128	112	1086	71	59	2173
18	152	325	255	67	175	703	57	23	1755
19	90	454	0	51	69	1237	7	20	1928
21	447	460	111	114	259	1052	32	12	2487
22	133	824	640	456	160	1983	356	82	4633
23	5329	9391	1768	2549	2065	22812	601	760	45274
24	6765	10311	1447	2555	2628	30158	702	716	55284
25	2439	4068	856	889	1118	11275	282	312	21239
26	3191	3899	1023	922	1197	10350	302	375	21259
28	1866	2559	760	595	822	6486	167	248	13502
29	267	866	100	85	96	2068	9	30	3522
30	778	1295	260	350	299	4850	8	104	7946
31	702	1178	486	268	379	3294	191	64	6561
32	1553	2648	472	772	677	8342	180	137	14781
Total ng/ml	35260	53227	11896	14147	14862	153011	4363	3983	290750
Average ng/ml	1410	2129	476	566	594	6120	175	159	11630

Total ARM 3									
No	Epigallocatechin	Gallocatechin Gallate	Epicatechin	Epicatechin gallate	Gallocatechin	Epigallocatechin Gallate	Catechin	Catechin Gallate	Total Catechins
4	14067	6045	4581	6711	2931	39345	1318	821	75819
6	4564	2170	1359	1921	1197	11123	483	419	23236
7	4467	2339	1414	2300	1024	9775	573	656	22548
8	7383	3210	2454	3341	1914	16852	883	726	36763
9	3107	2517	829	1528	936	8975	301	674	18865
10	2801	2664	929	1620	752	8184	359	489	17797
11	2501	2371	929	1299	668	7315	402	172	15657
13	3191	2509	1080	1272	852	7617	354	0	16875
15	2376	2262	809	1287	628	5877	339	415	13993
16	1829	1872	620	1023	628	4320	291	61	10645
17	975	1416	224	629	348	2860	143	0	6594
18	1656	1922	513	1083	518	3986	136	451	10265
19	761	1022	573	541	359	1734	260	20	5271
21	1367	1832	420	936	453	3932	320	0	9260
22	1231	1711	400	725	517	3523	259	0	8366
23	1224	1670	622	1295	502	3263	332	592	9501
24	1331	1451	444	983	468	3769	235	0	8682
25	1412	1503	651	1014	505	3756	222	0	9063
26	999	1327	393	584	448	2816	225	0	6791
27	1274	1376	438	731	466	3422	195	0	7902
28	984	1150	379	590	511	2560	171	0	6346
29	871	1043	324	539	443	1913	166	0	5299
30	2099	2408	905	755	755	5722	366	0	13010
31	829	983	819	1046	395	1536	368	0	5975
32	792	0	795	799	405	2034	302	0	5127
Total ng/ml	64090	48770	22905	34551	18624	166210	9005	5495	369649
Average ng/ml	2564	1951	916	1382	745	6648	360	220	14786



Graph 1 portrays the total catechin status of each of the participant after administration of the commercial investigation, the phytofare(R) and the Ph2 products.

The above results and graph clearly shows that the administration of the three products resulted in very different plasma status of the total catechins.



Graph 2 shows the comparative enhancement of the Phytofare^(R) and the Ph2 product against the commercial product used as a comparator in this study.

6. CONCLUSIONS

The following are the preliminary conclusions of the study:

- (i) That the plasma levels of catechins are substantially and statistically significantly enhanced in by the phytofare green tea extraction process as compared to that of a commercial green tea extract. The amount of average amount of total catechins found in the plasma of trial participants was 369 mg over 24 hours. That calculates to a total yield of 92% for the Ph2 product. This is of course not reasonable and one would expect that the high bioavailability to be partially the result of carry-over from the previous 24 hours, since the trial design prescribed the blood profiles were determined on the 4th day of administration of a 400mg dose. It is now quite important to calculate the corrected bioavailability.
- (ii) That the enhancement in plasma levels as a result of the phytofare extraction process were not equal for all the catechins analysed. **The enhanment ranged from 8 times to 62 times.** This large variation may in part be ascribed to the low values of catechins found in the commercial green tea extract for some of the catechins, most notably catechins gallate.
- (iii) That the plasma levels of catechins are substantially and statistically significantly enhanced in by the Ph2 formulation with selective advantages for specific catechins.
- (iv) The catechin found at the highest concentration for all three products was epigallocatechin gallate (EGCG). It is possible that saturation dynamics may come into play at very high absorption or distribution levels.
- (v) The higher plasma concentrations correlated with a higher number of adverse events or side effects in the case of phytofare but not Pheroid;
- (vi) The plasma levels observed after administration of the phytofare product did not return to zero within 12 hours of administration in the case of phytofare and 24 hours in the case of Ph2.. That resulted in an enhanced base line level from which the bioavailability parameters had to be calculated so that an overestimate of the enhancement in the bioavailability profile of the phytofare product is possible. However, in practice it would mean that the circulating half-lives of the catechins prepared according to the phytofare and especially the Ph2 are much longer and that a baseline level for catechins are maintained when using the current dosing intervals.

The above raise the concern that a dosage of 400mg a day is too high for both the phytofare-based products and may results in unacceptable adverse event profiles upon long term use.

However, the use of a 150 mg dosage of Ph2 once a day, in stead of 400mg, should result in a 3 x higher bioavailability for Ph2 than the bioavailability of the commerical product at a 400mg per day dosage.