Characterization of 6-paradol, 6-gingerol and 6-shagaol in AfperFit

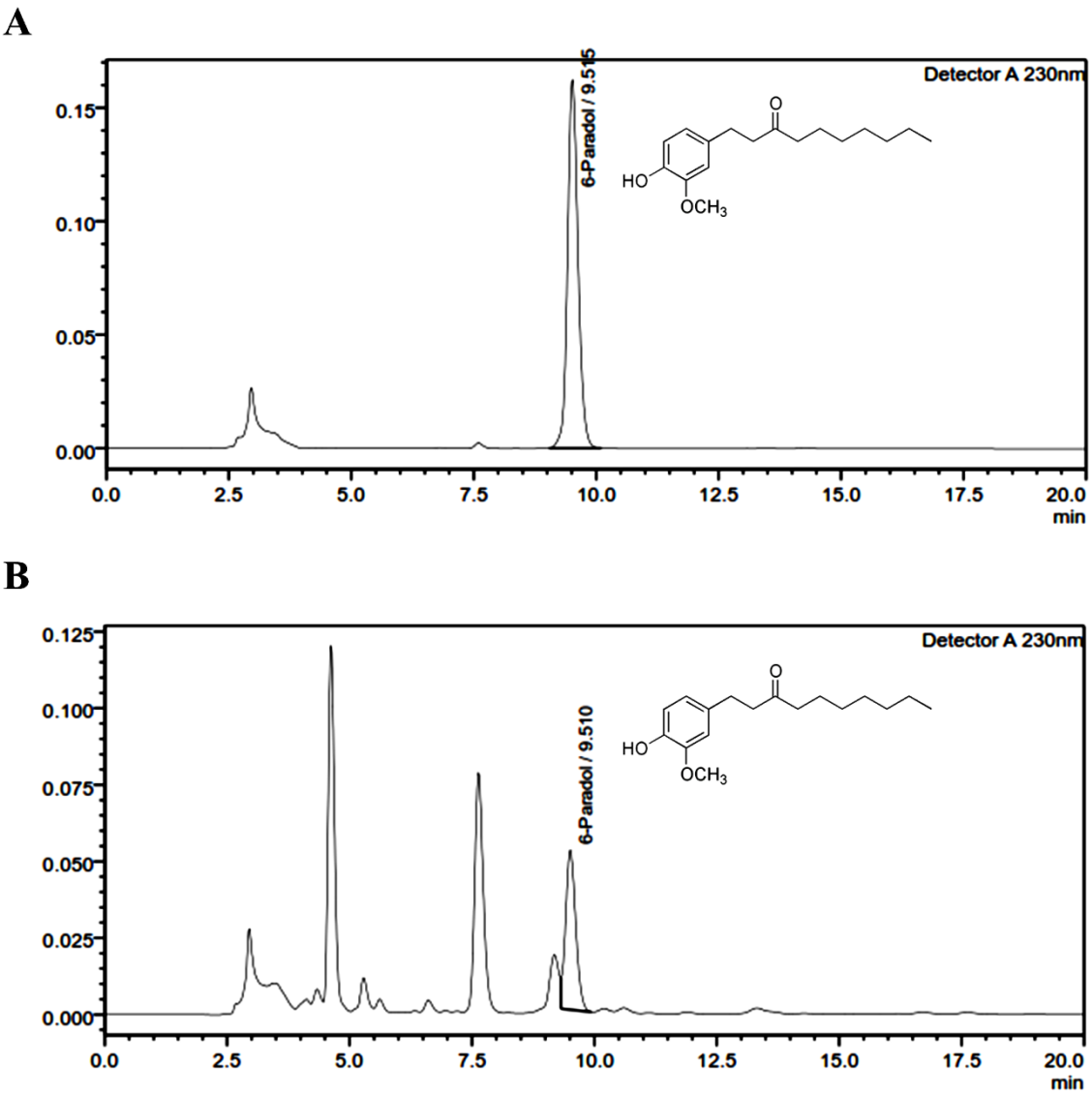
Materials

6-Paradol (HPLC grade, 98%) was procured from Chemfaces (China). 6-Gingerol (98 %) and 6-Shogaol (98 %) were procured from Sigma-Aldrich and China, respectively. All reagents and solvents were of analytical grade.

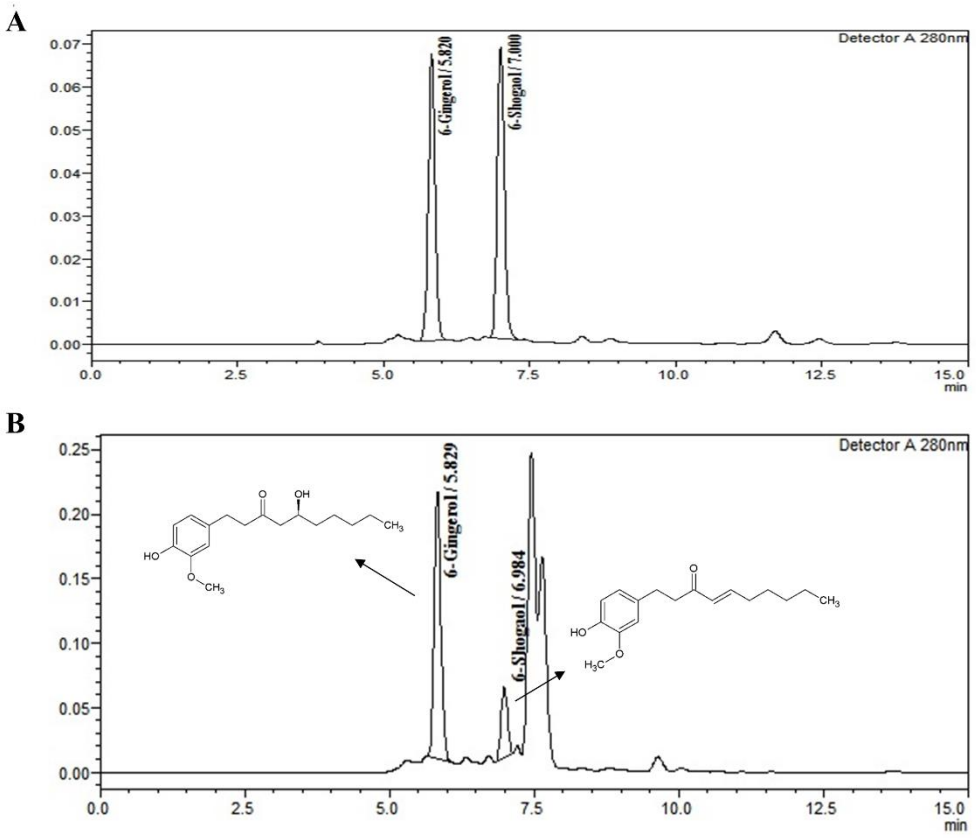
Method

HPLC was performed on a Shimadzu LC2030 C Prominence-i (Japan) system equipped with a quaternary low-pressure gradient solvent delivery LC2030 pump with high-pressure switching valves, online LC2030 degasser unit, a high sensitivity LC2030 ultraviolet (UV) detector, high speed drive LC2030 autosampler and large capacity column oven. The system controlled and data analyzed by LabSolutions software. A separation was carried out in Kinetex C-18 column (100 Aº, 250 mm × 4.6 mm, 5 μm pore size).

6-paradol in AfperFit was quantitatively analysed at UV detection of 230 nm using a mobile phase of a mixture of orthophosphoric acid (0.2%): acetonitrile (40:60) with isocratic flow rate of 1.2 ml/min and the injection volume of 5 μl. For the analysis of 6-shogaol and 6-gingerol contents in AfperFit a mobile phase of formic acid (0.5%): acetonitrile (10:90) with an isocratic flow rate of 0.5 ml/min was used. The compounds were quantified at UV detection of 280 nm. All solutions were degassed and filtered through 0.45 μm pore size filter. The column was maintained at 26 °C throughout analysis. Methanol was used as a diluent and the total liquid chromatography (LC) run time was 20 min. The peaks of compounds in AfperFit were quantified by comparing with the retention time (RT) of respective reference standards separately.



Supplementary Figure 1. HPLC chromatogram of 6-paradol. **(A)** Reference standard and **(B)** AfperFit



Supplementary Figure 2. **HPLC chromatogram of 6-gingerol and 6-shagaol. (A)** Reference standard and **(B)** AfperFit

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	01
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	01
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	02
	2b	Specific objectives or hypotheses	03
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	04
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	04
	4b	Settings and locations where the data were collected	04
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	04
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	05
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	04
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	05
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	05
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	05
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	05
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	05

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	03
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	05
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	N/A
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	05
	13b	For each group, losses and exclusions after randomisation, together with reasons	05
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	05
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	05
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	06-08
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	08
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	N/A
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	10
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	08 & 09
Other information			
Registration	23	Registration number and name of trial registry	03
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	10

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Supplementary Table 1. Study treatment details

	Treatment group A	Treatment group B
Product	AfperFit (*Aframomum melegueta* extract)	Maltodextrin
Dosage Form	Capsules	Capsules
Composition	Capsule weight – 250 mg AfperFit extract powder – 250 mg containing 5 mg of 6-paradol	Capsule weight – 250 mg Maltodextrin – 250 mg
Route	Oral	Oral
Dose	250 mg	250 mg
Dosing Regimen	Twice a day after food	Twice a day after food
Treatment duration	84 days	84 days
Manufacturer	Vidya Herbs Pvt Ltd.	Vidya Herbs Pvt Ltd.

Sample Size:

The sample size calculation was based on difference of 2 treatments are considered to be medically relevant. Assuming a common SD of 1.5 at the end of treatment, 35 per group would be sufficient to detect a difference of 1.1 in mean difference b/w the 2 treatment with power of 80% and a 0.05. 2-sided level of significance.

R-Program:

```
power.t.test(n=NULL, delta=1.1, sd=1.5, sig.level=0.05, power=0.80,
             type="two.sample", alternative="two.sided")

     Two-sample t test power calculation

              n = 30.18116
          delta = 1.1
             sd = 1.5
      sig.level = 0.05
          power = 0.8
    alternative = two.sided

NOTE: n is number in *each* group
```

A total of *N* number of subjects are required at each Treatment group in the end of the study with all the data being complete for analysis, but a proportion (q) are expected to drop out before the study ends. In this case, the following total number of subjects (*N1*) would have to be enrolled to ensure that the final sample size (N) in each Treatment group is:

$$N1 = \frac{N}{1-q} = \frac{31}{(1-0.10)} = 35$$

Where q is the proportion of attrition and is generally 10% in this type of studies.

Note: The proportion of eligible subjects who will refuse to participate (drop out) or provide the inadequate information will be unknown at the beginning of the study. Approximate estimates is often possible using information from similar studies.