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Chemical constituents of peels, kernels and hulls of fruits of *Mangifera indica* Var. Hiesy and their potential valorization.

Léa Herilala Rasoanaivo, Florian Albrieux, Marc Lemaire

Abstract
In the aim of valorization of the by-products of fruit processing of *Mangifera indica* Var. Hiesy, separation and phytochemical study were performed.
Fatty acids and phytosterols of kernel oil of *Mangifera indica* Var. Hiesy are characterized by Gas Chromatography coupled with mass spectrometry or flame-ionization detector**.**
Separation method is based on ethanolic extract and then organic –aqueous partition extract using organic solvent in increasing polarity. Purification of gallic acid and its esters was made by precipitation with ethanol 99 °C.
Polyphenolic compounds such phenolic acids; gallotannins and flavonoid are identified and quantified in ethanolic extract of by-products (wastes) of fruits processing of *Mangifera indica* Var. Hiesy. Identification of polyphenol compounds has been established by high-performance liquid-chromatography-electrospray ionisation mass spectrometry and nuclear magnetic resonance spectrometry.
Content in phenolic compound and tannin are given. The potential utilizations of metabolite separated are discussed. Saponification then acidification of extract rich in gallotannins gives gallic acid using in synthetic chemistry.

Keywords: *Mangifera indica* L. Var. Hiesy / *Anacardiaceae* / Madagascar / gallotannins / oil / phenolic acids / valorization.

1. Introduction
Mangifera indica L. (Anacardiaceae), fruit tree origins of the East of India and Birmane had over 500 varieties in the world [1]. This species is also represented in Madagascar by 46 varieties [2] with 205000 tons products each year [3]. In Madagascar, fruit of *Mangifera indica* L. is popularly known as "manga" followed by variety name such "hiesy" or "vato" or "Zanzibar". According to our ethnobotanical investigations led with population and traditional healers in the region, manga kernel is traditionally used to treat parasite. After consumption and industrial processing of the fruits of manga, considerable amounts of manga peels, seeds are discarded as wastes. View the amount of peels and seeds of mangoes after their transformation into juice industry, chemistry and processing of these materials are the subject for several studies in the recent years. Peels, kernels recovery in various varieties of *Mangifera indica* allow for polyphenolic compounds [4]: phenolic acids, xanthones, flavonoids, and tannins [2]. Tannins extract of kernel have antimicrobial [6, 7, 8], antioxidant, anti-snake venom, hepatoprotective, anti-tyrosinase, inhibitory activity [9, 10, 11, 12]. Oils of kernel in many varieties of *Mangifera indica* L. are characterized [13, 14, 15]. Several valorizations of metabolite from kernel and peel were proposed [16]: starch of kernel to biopolymer [17]; peel and kernel to additive of biscuit [4], kernel oil to cocoa butter in cosmetic [19], proteins and starches for feeding the animals.
Madagascar is an island rich in tropical fruits, the valorization of by-product of a fruit processing may be an economical source.
In this study, the identification and quantification of kernel oil and polyphenolic compounds in kernel, hull and peel of fruit of *Mangifera indica* L. var. Hiesy, known as vernacular name "manga hiesy" or "manga hoesy" in Madagascar, are reported. The objective was to evaluate a possible utilization of separated fractions of these by-products of fruits processing.

2. Materials and methods

2.1. Plant material

Fruits of "manga hiesy" with a green peels were collected by Hervé Andriamadio in september 2013 in Antsohihy district on the road RN6 in North of Madagascar.

2.2. Chemicals and General experimental procedures

Solvents used were purchased from VWR (Darmstadt, Germany) and were of gradient grade or analytical. Deionised water was prepared by a milli-Q water purification system (Millipore, France). Gallic acid and reagents were obtained from Sigma (Steinheim, Germany). Skin pre-chromed powder was furnished with SCRD group "Compagnie Française des extraits" (LeHavre, France). Thin layer chromatography was performed on precoated TLC plates (Merck, silica $60F_{254}$) and visualized by UV light and by spraying with vanillin in H_2SO_4 or with $FeCl_3$ in CH_3OH. 1D (^1H, ^{13}C, DEPT) and 2D (^1H-^1H COSY, ^1H-^{13}C HSQC, ^1H-^{13}C HMBC) NMR spectra were recorded on a Bruker Varian 400 NMR operating at 400.15/125.15MHz using, CD_3OD as solvent and TMS as an internal standard. HPLC/MS analysis was carried with Agilent HPLC G1312A binary gradient pump series system equipped with HYSTAR software, a model G1329A autosampler. The HPLC system was connected in series with a Micro TOF-Q Bruker Daltonics (Bremen, Germany) mass spectrometer fitted with ESI source. Negative ions mass spectra of the column eluate were recorded in the range m/z 50-2000 (Resolution 10 000). Nitrogen was used as the drying gas at a flow rate 8L/min. The nebulizer temperature was set at 200 °C and the pressure is 2 bars. The gas chromatograph (GC–MS) was performed by using a Focus GC instrument (Thermo Electron Corporation, Bremen, Germany) equipped with a DB-5 MS capillary column (30 m, 0.25 mm inner diameter, 0.25 mm film thickness) and the mass analyzer was a simple quadrupole with an electron ionization system was used with an electron energy of 70 eV.

The carrier gas was He with a flow rate of 1 mL/min. The column temperature was initially 70 °C for 2 min, which gradually increased to 310 °C at 15 °C/min and was finally kept at 310 °C for 10 min. The injector temperature was 220 °C, and the transfer line temperature was 280 °C.

The gas chromatograph (GC-MS) was used for separation of methyl ester (or sterol sylilated). The fatty acids methyl esters were identified by comparison of their corresponding mass in MS library.

Percent relative fatty acid (or phytosterol) was calculated based on the peak area of a fatty acid (or phytosterol) species to the total peak of all the fatty acids (or phytosterol) in the oil sample using GC-2025 Shimadzu equipped with flame-ionisation detector (GC/FID) and a polar capillary column (ZB-5MS) 0.25 mm internal diameter, 30 m length and 0.25 µm film thickness. Before injection, solutions of product or extract were filtered with 0.45 µm nylon 66 membrane filter (Supelco).

2.3. Extraction and isolation of the constituent

Pulp, peel, kernel and hull of fruits (2250 g) are separated. The waste 50.5% w/w (1136.25 g; peels, kernels, hulls) of the fruits of "manga hiesy" was kept at room temperature (25-30 °C) for air drying

(3 weeks). The air-dried powder 221.1 g (9.8%, w/w) of peels; 241.9 g (10.8%, w/w) of hulls and 173.4 g (7.7%, w/w) of kernels were used as material.

2.3.1 Oil kernel extract

20.0 g of powder kernels were extracted with hexane by soxhlet at 80 °C (3 x 200 ml, 8 h each). The solvent was dried over anhydrous sodium sulphate. The filtered solvent was evaporated under vacuum to afford a lipid pale yellow semisolid (1.4 g, 6.1% w/w) and a defatted kernel (18.3 g, 91.6% w/w).

2.3.1.1. Transesterification of fatty ester

25.0 mg of oil were heated with 1ml BF_3-methanol and 1 ml 2, 2-dimethoxypropane reagent in reflux at 60 °C for 15 min [4]. The esters were extracted with hexane. The extracts were dried over anhydrous sodium sulphate, filtered and the solvent was removed to get (20.4 mg, 81.6% w/w) of the methyl esters of fatty acids.

2.3.1.2 Unsaponifiable extraction

423.8 mg of fat was refluxed with 15 ml of 0.5 N ethanolic potassium hydroxide during 4 hours for saponification. After filtration, the ethanol was evaporated and then water and diethyl ether were added. The upper ethereal layer was dried over anhydrous sodium sulphate and distilled of to get (12.8 mg, 3% w/w) unsaponifiable matter.

The unsaponifiable fraction is introduced into a test tube in which silylation reagent (0.5 ml of pyridine, 0.1 ml of hexamethyldisilazane and 0.04 ml of trimethylchlorosilane) are successively introduced [21]. After maceration in oil bath at 60 °C for 5 min, the mixture was decanted. The supernatant is diluted with hexane then filtered before injection into the column.

2.3.2 Ethanolic extract in peel, hull and defatted kernel

40.0 g of peels, 24.3 g of hulls and 53.0 g of defatted kernels were extracted respectively three times in ultrasonic apparatus at 30 °C with mixture of ethanol water 80:20 (3 x 200 ml, 30 min each). The filtered solvent was evaporated under vacuum to obtain respectively 12.3 g (30.6%, w/w) green, 5.8 g (23.9%, w/w) green and 12.5 g (23.6%, w/w) red solid gum. The identification of constituents is obtained after purification of ethanolic extract.

2.3.2.1 Separation of ethanolic extracts

893.3 mg of ethanolic extract of the kernel was treated with ethanol 99 °C to give precipitates 67.2 mg (7. 4% w/w) rich in tannins noted product P. Product P is used for analysis in 1D NMR and 2D NMR and LC/MS ESI of tannin constituents of kernel in "manga hiesy".

Residue ethanolic of peel (12.3 g), hull (5.8 g) and kernel defatted (6.2 g) was suspended respectively in hot water (40 °C) and filtered in warm to remove the chlorophyll. The obtained extractive solution was respectively partitioned by successive extractions three times with different solvents of increasing polarity to yield hexanic (peel 3.4 g: 28.0%; defatted kernel 0.0012 g: 0.02% ; hull 0.09 g: 1.7%), methylene chloride (peel 0.36 g: 2.94%; defatted kernel 0.012 g: 0.20%; hull 0.013 g: 0.22%), ethyl acetate (peel 4.44 g: 36.27%; defatted kernel 2.23 g: 36.00%; hull 0.96 g: 16.55%) and aqueous (peel 4.00 g: 32.6%; defatted kernel 3.90 g: 62.75%; hull 4.63 g: 79.84%) fractions.

AcOEt extracts of peel and hull are used respectively for identification of polyphenolic by comparison of retention time and mass with kernel constituent.

Gallotannins in ethyl acetate extracts of peel, hull and kernel were saponified with NaOH 10 N then acidified with H_2SO_4 to

give gallic acid.

2.3.2.2 Analysis of polyphenol in fractions of crude ethanolic extracts of "manga hiesy" by HPLC with MS ESI.

Chromatography was carried out on an EC 125/3 NUCLEODOR C18 Polar Tec column (125 mm x 2mm, 3 µm particle size) from Macherey-Nagel (Germany), at room temperature using solvent A (0.01% v/v formic acid in water) and solvent B (acetonitrile) with a gradient program: 10% B to 17.5% B (25 min), 17.5% B to 20% B (25 min), 20% B to 40% B (20 min) and equilibration in 2 min. Flow rate and injection volume were 0.3 ml/min and 4 µl, respectively. Precipitates of ethanolic extract of kernel and ethyl acetate fractions of peel and hull were obtained from "manga hiesy" according to the protocol described in part 2.3.2.1. Each extract (4 mg/ml) was dissolved in methanol: water (20:80) for qualitative determination of constituents. The selectivity of the method was determined by analysis of standard compound and samples. The peak of gallic acid was identified by comparing its retention time with those of the standard and the mass spectrum.

2.3.3 Aqueous acetone extract of peel, hull and defatted kernel

To evaluate the polyphenolic content, peel (2.5 g), hull (3.2 g) and defatted kernel (5.0 g) of "manga hiesy" were extracted respectively with acetone / water 70/30 in ultrasonic for 30 minutes

(3 times) to give aqueous acetone extracts of kernel (1.7672 g, 35,34%w/w) , peel (0.6225 g, 24.9%w/w), hull (0.5439 g, 21,7%) and a residue of kernel (3.2328 g, 64.656% w/w) , peel (2.5775 g, 80.54% w/w) , hull (1.9561 g, 78,2% w/w).

2.3.4 Determination of total polyphenol content in aqueous acetone and ethanol extracts of by-product of fruit processing of "manga hiesy"

Total polyphenol content was measured by the Folin–Ciocalteu method with slight modification [22]. An aliquot of 0.5 ml of sample solution (with appropriate dilution to obtain absorbance in the range of the prepared calibration curve) was mixed with 2.5 ml of Folin–Ciocalteu reagent (10 times dilution). After two minutes, 2.0 ml of saturated Na_2CO_3 solution (145 g/L) was added. The mixture was allowed to react at 50 °C for 5 min, then to cool and stand 5 min before the reading of absorbance of the reaction mixture at 760 nm.

A calibration curve of gallic acid (ranging from 0.01 to 0.10 mg/ml) was prepared, and total polyphenolic content was standardised against gallic acid and expressed as mg gallic acid equivalent per gram of sample on a dry weight basis.

2.3.5 Quantification of hydrolysable tannin in aqueous acetone extract of "manga hiesy"

The tannin content was determined by the official hide-powder method [23]. Aqueous solutions of concentration 4g / l (1 g/ 250 ml) were prepared from these samples, using hot water (40 ° C). 10 ml of each extract were evaporated and then dried in an oven at 100 °C until a constant weight to afford a mass of extract A. Twenty milliliter of each extract was filtered on filter paper three times until a clear solution. Ten milliliter of the filtrate were evaporated and dried in the same condition to give the mass of soluble extract B. Hundred milliliter of each sample are introduced into a 250 ml Erlenmeyer flask containing 3.5 g of skin powder pre-chromed then magnetically stirred for ten minutes. After filtration on Buchner, 10 ml of the limpid filtrate were evaporated and dried under the same conditions as above to give a mass of untannin solution C. The difference between the soluble extract mass (B) and the mass of extract untannin (C) gives the tannin content of the extract.

3. Results and discussion

3.1. Oil kernel

The oil content of "manga hiesy" kernel (6, 1%) is low compared to literature data; 31% of oil kernel was found using a mixture of chloroform methanol as solvent [9]. Variation in oil yield may be due to the difference cultivation climate, ripening stage, the harvesting time of the seeds kernel and the extraction method used. The GC/MS of oil kernel of "manga hiesy" is given in figure 1.

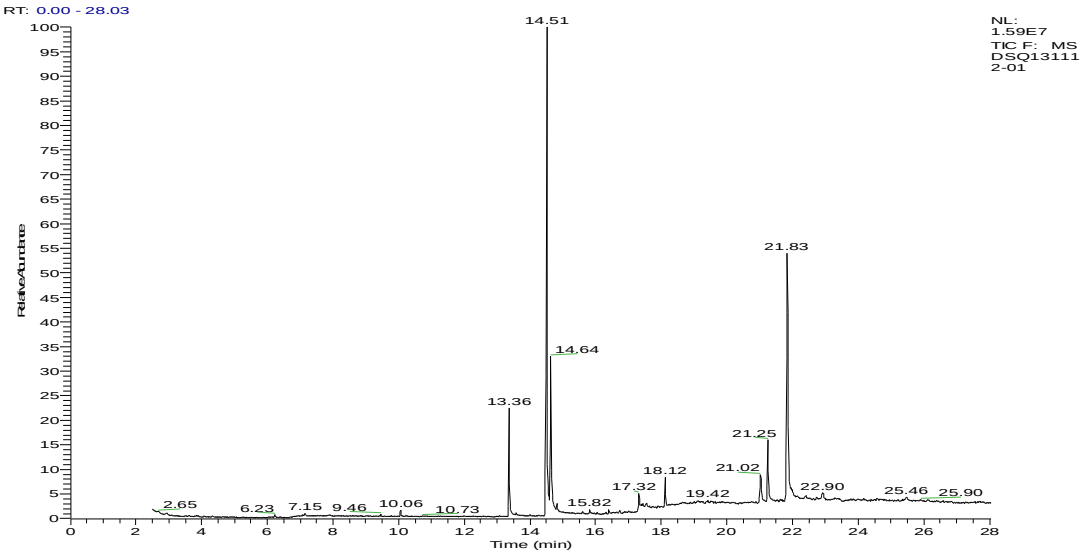

Fig 1: Separation of fatty acids and sterols in manga hiesy kernel oil by GC. Peak assignment: tr= 13.36: palmitic acid; tr=14.50: linoleic acid; tr=14.51:oleic acid, tr=14.64:stearic acid, tr=21.02:campesterol, tr=21.25:stigmasterol, tr =21.83 β-sitosterol.

3.2 Fatty acid composition of kernel oil

The major unsaturated fatty acids in "manga hiesy" kernel oil (6.1% of kernel) were oleic (46.22%) and linoleic (7.33%) acids and the main saturated fatty acids are stearic (8.72%),

palmitic (37.73%).

3.3 Phytosterol composition of kernel oil

The three major phytosterol (3% of kernel) detected in "manga hiesy" kernel (Figure 2) are campesterol (19.5%), stigmasterol (25.0%) and β-sitosterol (55.5%). The constituents of oil kernel in "manga hiesy" are consistent with literature data (Gaydou *et al.*, 1984). This result showed that oil kernel of "manga hiesy" can be used in cosmetic industry and it is a source of phytosterol used as a dietary supplement to prevent cardiovascular disease [25].

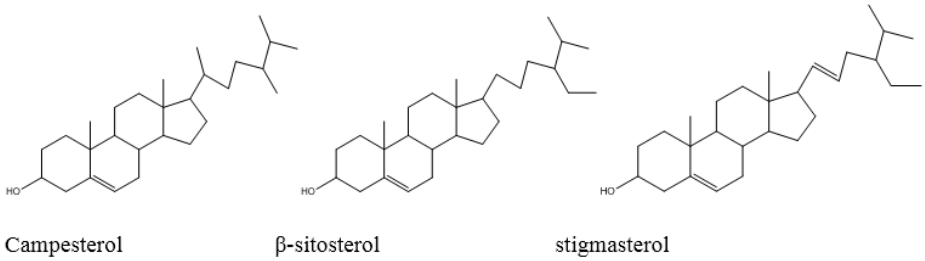

Fig 2: Phytosterol in kernel oil of "manga hiesy".

3.2 Constituents of peel, hull and kernel of "manga hiesy"

3.2.1. Tannin and phenolic acid composition of ethanolic extract of kernel

A fraction noted P (1.74%) of "manga hiesy" kernel powder was prepared by precipitation with ethanol 99° of ethanolic extract. The separation by HPLC of this fraction from kernel is shown in Figure 3.

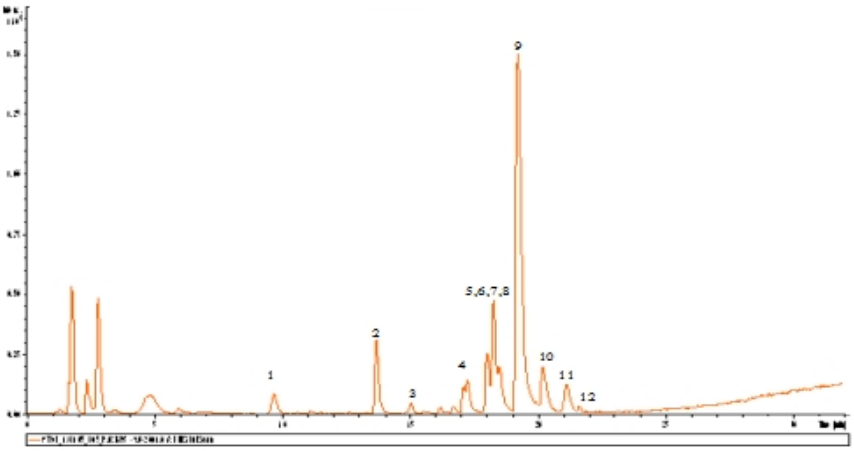

Fig 3: Separation of phenolic acids and gallotannins in "manga hiesy" kernel by HPLC. Peak assignment: (1) sodium gallate, (2) methyl gallate, (3) tri-O-galloyl-glucoside, (4, 5, 6, 7, 8) five isomers of tetra-O-galloyl-glucoside, (9) penta-O-galloyl-glucoside, (10) hexa-O-galloyl-glucoside, (11) hepta-O-galloyl-glucoside, (12) octa-O-galloyl-glucoside.

Phenolic acids

Three phenolic acids were detected in the ethanolic fraction of kernel. Compound 1 showed a $[M-H]^-$ ion of m/z 191 identified as sodium gallate by ESI mass spectrometry. Compound 2 showed a $[M-H]^-$ ion of m/z 183 identified as methyl gallate. The third compound had a $[M-H]^-$ ion of m/z 197 and identified as ethyl gallate is a compound 4. It is a major product of P fraction. Ten milligrams of ethyl gallate (total yield 5%) had been isolated from ethyl acetate fraction of kernel. The signal characteristics of galloyl moiety (G5 and G4 of phenolic acids in table 3), ethoxy group (1.32 ppm /18.3 ppm; 4.24 ppm /58.24 ppm) and methoxy group (51.6 ppm / 3.89 ppm) were observed in NMR spectra of fraction P [26].

Gallotannins

Ten gallotannins were identified in kernel using mass spectra. They are consisted of glucose and three to nine gallic acids moieties. The NMR spectra of kernel fraction exhibited many peaks for gallotannins [27, 28]. Data NMR of glucose and galloyl moiety in gallotannins of kernel fraction is presented in tables 1 and 2 respectively.

Table 1: Data NMR of glucose moiety in gallotannins of the kernel of manga hiesy in CD_3OD

Glucose moiety	δ_{1H} (ppm)	δ_{13C} (ppm)
CH	5.75	93.8
CH	3.77	73.28
CH	4.1	74.37
CH	3.63	71.39
CH	3,83	75.9
4CH_2	3.25, 3.41, 3.50, 3.51	63.25, 62.39, 60.85, 60.84

Table 2: Data NMR of galloyl moiety of hydrolysable tannins and esters of gallic acid in ethanolic extract of kernel of "manga hiesy" in CD_3OD (δ ppm)

Position	δ (^{13}C)ppm G1[a]	δ (^{13}C) ppm G2[a]	δ (^{13}C) ppm G3[a]	δ (^{13}C) ppm G4[a]	δ (^{13}C) ppm G5[a]	δ (^{13}C) ppm G6[a]
-O-C=O	165.9 (1C)	165.5(1C)	165.4(1C)	164.7(1C)	167.6 (1C)	166.5 (1C)
1	118.8(1C)	118.7(1C)	118.5(1C)	118.7(1C)	119.9(1C)	119.5(1C)
2, 6	6.92[b](2H) 108.8(2C)	6.97[b](2H) 108.8(2C)	6.99[b](2H) 108.9(2C)	7.06[b](2H) 108.0(2C)	7.07[b](2H) 107.1(2C)	7.13[b](2H) 108.7(2C)
3	144.7(1C)	144.8(1C)	144.9(1C)	145.1(1C)	145.1(1C)	144.9(1C)
4	138.6(1C)	138.6(1C)	139.0(1C)	139.2(1C)	138.3(1C)	138.7 (1C)
5	144.7(1C)	144.8(1C)	144.9(1C)	145.1(1C)	145.1(1C)	144.9 (1C)

[a] galloyl moiety group
[b] chemical shift of proton in galloyl moiety

Eight types of galloyl moiety for gallotannins and phenolic acids are observed in kernel fraction. Examples of polyphenol structure detected in ethanolic extract of "manga hiesy" kernel are presented in Figure 4.

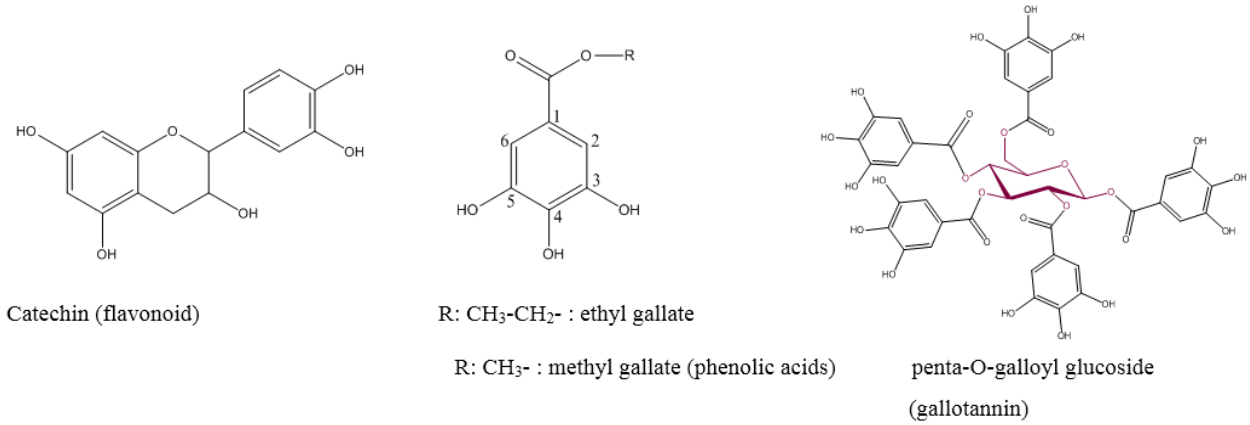

Fig 4: Examples of polyphenol structure in "manga hiesy" kernel.

Phenolic acids and gallotannins identified in kernel are given in table 3.

Table 3: Characteristic ions of phenolic acids and gallotannins from defatted kernel extract of *Mangifera indica* L. Var. Hiesy

N° peak	Retention times (mn)	Identified compounds	[M-H]$^-$
1	9.7	Sodium gallate	191
2	13.7	Methyl gallate	183
3	15.0	Tri-O-galloyl-glucoside	635
4	17.2	Tetra-O-galloyl-glucoside	787
5 to 8	17.2-18.2 (18.2)	four isomers of tetra- O-galloyl-glucoside	787
9	19.1	Penta- O-galloyl-glucoside	939
10	20.3	Hexa- O-galloyl-glucoside	1091
11	21.1	Hepta- O-galloyl-glucoside	1243
12	21.6	Octa- O-galloyl-glucoside	1395

The fraction containing mainly gallic acid and ester represented 7.4% of ethanolic extract ie 1.74% of kernel.

3.2.2. HPLC/MS ESI analysis of ethyl acetate extract of "manga hiesy" peel

The separation of AcOEt extract from "manga hiesy" peel is shown in figure 5.

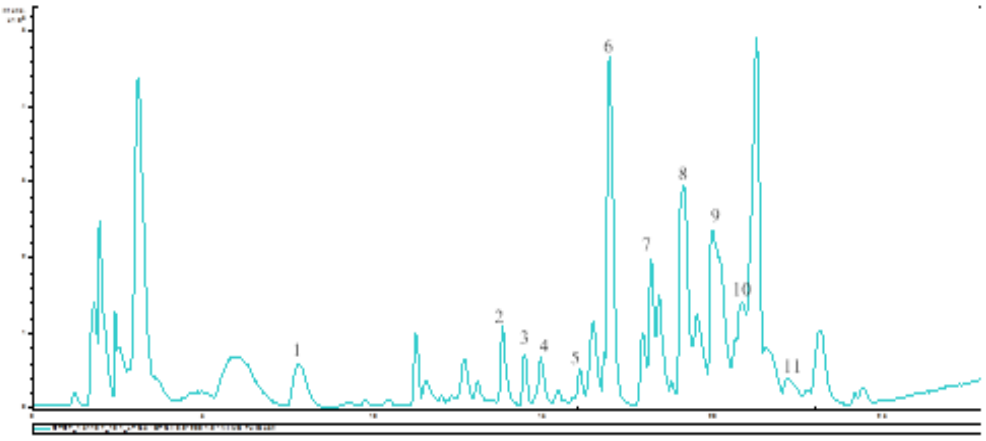

Fig 5: Separation of phenolic acids, flavonoid and gallotannins in "manga hiesy" peel by HPLC. Peak assignment: (1) gallic acid, (2) di-O-galloyl-glucoside, (3) catechin (4, 5) two isomers of tri-O-galloyl-glucoside, (6) ethyl gallate, (7) three isomers of tetra-O-galloyl-glucoside, (8) penta-O-galloyl-glucoside, (9) hexa-O-galloyl-glucoside, (10) hepta-O-galloyl-glucoside, (11) nona-O-galloyl-glucoside.

Phenolic compounds are identified as phenolic acid, flavonoid and gallotannins by their mass and compared of constituents of kernel, which are given in table 4.

Table 4: Characteristic ions of phenolic acid, flavonoid and gallotannin from peel extract of *Mangifera indica* L. Var. Hiesy

N° pics	Retention time (mn)	Identified tannin	$[M-H]^-$
1	7.8	Gallic acid	169
2	11.3	nd	443
3	13.8	Di-O-galloyl-glucoside	483
4	14.5	catechin	289
5	14.9	Tri-O-galloyl-glucoside	635
	16.1	Tri-O-galloyl-glucoside	635
6	17.0	Ethyl gallate	197
7	18.2	Tetra- O-galloyl-glucoside (four isomers)	787
8	19.1	Penta- O-galloyl-glucoside	939
9	20.0	Hexa- O-galloyl-glucoside	1091
10	20.8	Hepta- O-galloyl-glucoside	1243
11	22.2	Nona- O-galloyl-glucoside	1548

Two phenolic acids are identified: gallic acid 1 and ethyl gallate 6. Based on the identification above compounds 3 and 5-11 could be attributed as di, tri-, hexa-, hepta-, octa- and nona-O-galloyl-glucoside respectively.

The fraction (ethyl acetate extract of peel) containing mainly gallic acid and esters represented 36.28% of ethanolic extract ie 11.10% of the peel.

3.2.3. HPLC/MS ESI analysis of ethyl acetate extract of "manga hiesy" hull

The separation of AcOEt extract from "manga hiesy" hull is shown in Figure 6.

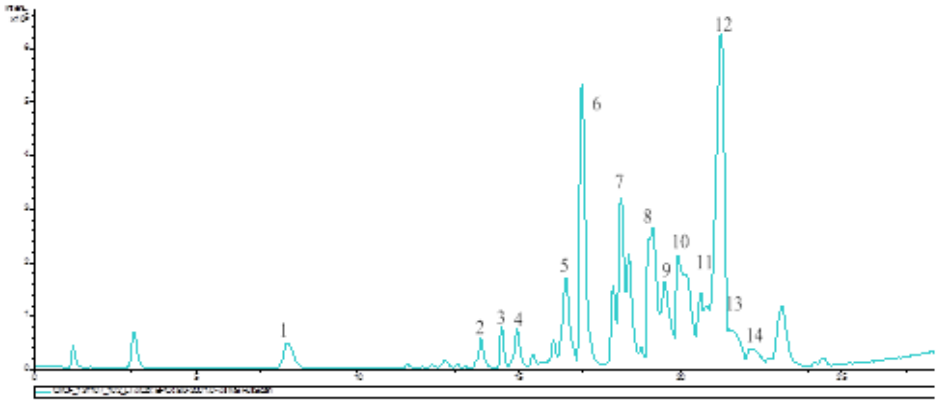

Fig 6: Separation of phenolic acids, flavonoid and gallotannins in "manga hiesy" hull by HPLC. Peak assignment: (1) gallic acid, (2) di-O-galloyl-glucoside, (3) catechin (4, 5) tri-O-galloyl-glucoside,(6) ethyl gallate, (7) three isomers of tetra-O-galloyl-glucoside, (8, 9) penta-O-galloyl-glucoside, (10) hexa-O-galloyl-glucoside, (11, 12) hepta-O-galloyl-glucoside, (13) octa-O-galloyl-glucoside, (14) nona- O-galloyl-glucoside.

Phenolic compounds are identified as phenolic acid and gallotannins by their mass and comparison of data in kernel constituents, which are presented in table 5.

Table 5: Characteristic ions of phenolic acid, flavonoid and gallotannin from hulls extract of *Mangifera indica* L. Var. Hiesy

N° pics	Retention time (mn)	Identified compound	$[M-H]^-$
1	7.8	Gallic acid	169
2	13.8	Di-O-galloyl-glucoside	483
3	14.5	Catechin	289
4	14.9	Tri-O-galloyl-glucoside	635
5	16.1	Tri-O-galloyl-glucoside	635
	16.5	Nd	321
6	16.9	Ethyl gallate	197
7	17.9	Tetra- O-galloyl-glucoside (four isomers)	787
8	19.2	Penta- O-galloyl-glucoside	939
9	19.6	Penta- O-galloyl-glucoside	939
10	19.9	Hexa- O-galloyl-glucoside	1091
11	20.6	Hepta- O-galloyl-glucoside	1243
12	21.3	Hepta- O-galloyl-glucoside	1243
13	21.8	Octa- O-galloyl-glucoside	1395
14	22.2	Nona- O-galloyl-glucoside	1548

Constituents of hull were identified by comparison of the chromatogram of peel and kernel. Other gallic acid (compound1) , di-O-galloylglucoside (compound 2), catechin (compound 3) and ethyl gallate (compound 6), compounds 4, 5 and 7-14 could attributed respectively to tri, tetra- (four isomers), penta-, hexa-, octa- and nona-O-galloylglucoside.

The fraction ethyl acetate extract of hull containing mainly flavonoid, gallic acid and ester represented 16.55% of ethanolic extract and 4.06% of hull.

Peels, kernels and hulls of "manga hiesy" contain gallotannins such the other varieties of *Mangifera indica* [29, 30]. Tri- to nona-O-galloylglucoside were identified using LC/MS ESI and NMR spectra.

3.3. Polyphenolic content

The percent of total polyphenolic content expressed as mg gallic acid equivalent per gram of sample on a dry weight are given in table 6.

Table 6: Determination of total polyphenol content of crude extracts using Folin-Ciocalteu method

Crude extract	Peel (%)	Kernel (%)	Hull (%)
Ethanolic	22.8	35.7	11.3
Hydroacetonic	37.2	42.7	13.6

The Folin-Ciocalteu reagent is used to obtain a crude estimate of the amount of phenolic compounds present in an extract. Different crude extracts of kernel, peel and hull (ethanolic, and hydroacetonic (70/30) were prepared in order to compare their total phenolic content. As results, total phenolic content of the extracts decreased in the following order, as shown in Table 6: hydroacetonic extract of kernel > hydroacetonic extract of peel > crude ethanolic extract of kernel > crude ethanolic extract of peel > hydroacetonic extract of hull > crude ethanolic extract of hull. The total phenolic content of hydroacetonic extracts (kernel, hull, peel) was higher than those of crude ethanolic extracts. The lower polyphenol value was found in crude ethanolic extract of hull.

3.4. Content tannin

The amount in hydrolysable tannin of aqueous acetone in peel, kernel and hull in "manga hiesy" are shown in table 7.

Table 7: Tannin content of hydroacetonic extract in peel, hull and kernel of "manga hiesy"

Hydroacetonic extract	Peel	Kernel	Hull
Tannin content (%)	15,7	29,6	12,0

The higher tannin value was found in crude hydroacetonic extract of kernel. Hydroacetonic extract of kernel seems to be rich in tannin. Its result is in agreement with the literature data [31].

4. Conclusion

Results obtained showed that "manga hiesy" kernel powder contained 0.5 % moisture, 6,1% crude oil (hexane), 10.44% gallotannins, 4.63% other polyphenol compounds, and 64.25 % residual containing protein and starch.

Based on the above reviews, it could be concluded that the "manga hiesy" kernel, hull and peel could be used as a potential source for bimolecular: polyphenolic (natural antioxidants), gallotannin (antimicrobial compounds), sterol (cosmetic), in addition, it could be further processed into therapeutic functional food products. Saponification of gallotannins furnished an amount quantity of gallic acid after acidification. This suggests that the kernel, hull and peel should be further utilized rather than just discarded as waste. From an economic point of view, it seems that this valorization can be envisaged only if all the valuable compounds are separated and used. An abstract of valorization method is given in Figure 7. The total percent of gallotannins and other polyphenol compounds of by-products of "manga hiesy" fruits processing is given.

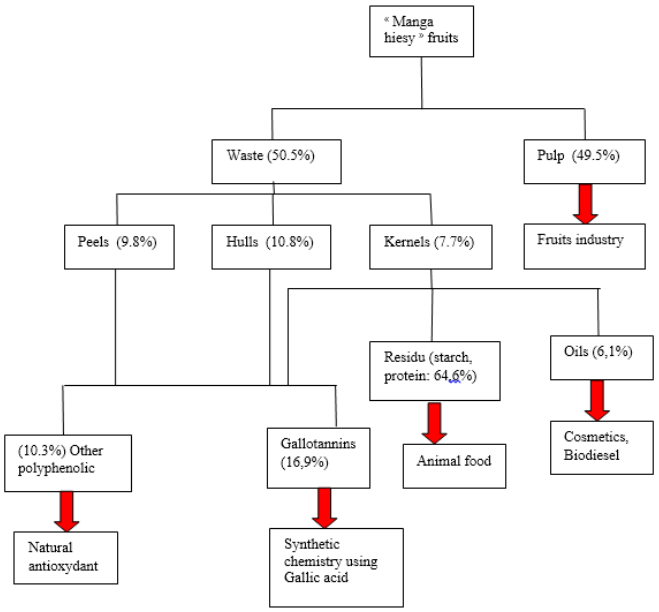

Fig 7: Valorization method of kernel, hull and peel of *Mangifera indica* L. Var. Hiesy fruits.

Therefore, the utilization of by-products of "manga hiesy" fruits processing especially kernel, hull and peel, may be an economical way to reduce the problem of waste disposal from "manga" production in Madagascar. We can obtain 61 g oil rich in phytosterol (3%) with 1 kg of kernel. The extracts of peel, hull and defatted kernel exhibited a high phenolic content due to the occurrence of important amounts of gallotannin. This is probably the main interest of such waste. In 1kg of by-product of "manga hiesy" fruit processing, 169.4 g of gallotannins can transformed into gallic acid using in organic synthesis and 103.4 g other phenolic compounds will be employed such natural antioxidant. Moreover only very few aromatic derivatives are available from natural renewable sources gallic acid had aromatic with four oxygenated functional groups. This molecule may be transform into numerous useful reagents and intermediates using relatively simple chemistry and we work now on the transformation of the obtained gallic acid. As far as we knew, composition of "manga hiesy" peel, hull and kernel and related products have not been previously characterized in detailed knowledge as we do. This composition is of interest as far as renewable starting material is sought.

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6. References

1. Nathalie W, Aliou B, Elhadj SB, Marc VD, Pierre D. Ethnopharmacology of *Mangifera indica* L. Bark and Pharmacological Studies of its Main C-Glucosylxanthone, Mangiferin. International Journal of Biomedical and Pharmaceutical Sciences © Global Science Books 2007; 113-119.
2. Zenaivo A. Catalogue des variétés de mangues dans la région du Boeni, CENRADERU – FOFIFA 2003; 8.
3. FAOSTAT (2012). FAO statistical Database. Agricultural data http://193.43.36.221/DesktopDefault.aspx?PageID=567&lang=fr#ancor 29 April, 2014.
4. Barreto JC, Trevisan MT, Hull WE, Erben G. de Brito E, S, Pfundstein B *et al.* Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). J Agric Food Chem 2008; 56(14):5599-5610.
5. Schieber A, Berardini N, Carle R. Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv, "Tommy Atkins") Peels by High-Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry 2003; JF030218F.
6. Engels C, Knödler M, Zhao YY, Carle R, Gänzle MG, Schieber A *et al.* Antimicrobial activity of gallotannins isolated from mango (*Mangifera indica* L.) kernels. J Agric Food Chem 2009; 57(17):7712-7718.
7. Engels C, Gänzle MG, Schieber A. Fractionation of Gallotannins from mango (*Mangifera indica* L.) kernels by high-speed counter-current chromatography and determination of their antibacterial activity. J Agric Food Chem 2010; 58(2):775-780.
8. Arogba SS. Mango (*Mangifera indica*) kernel: Chromatographic analysis of the tannin, and stability study of the associated polyphenol oxidase activity. Journal of food composition and analysis 2000; 13(2):149-156.
9. Pithayanukul P, Leanpolchareanchai J, Saparpakorn P. Molecular docking studies and anti-snake venom metalloproteinase activity of Thai mango seed kernel extract. Molecules 2009; 14(9):3198-3213.
10. Nithitanakool S, Pithayanukul P, Bavovada R. Antioxidant and hepatoprotective activities of Thai mango seed kernel extract. Planta Med 2009a; 75(10):1118-1123.
11. Nithitanakool S, Pithayanukul P, Bavovada R, Saparpakorn, Molecular docking studies and anti-tyrosinase activity of Thai mango seed kernel extract. Molecules 2009b; 14(1):257-265.
12. Pimsumon J, Pimolpan P, Rapepol B, Mullika TC. The Inhibitory Potential of Thai Mango Seed Kernel Extract against Methicillin-Resistant *Staphylococcus aureus.* Molecules 2011; 16:6255-6270.
13. Nzikou JM, Kimbonguila A, Matos L, Loumouamou B, Pambou-Tobi NPG, Ndangui CB *et al.* Extraction and Characteristics of Seed Kernel Oil from Mango (*Mangifera indica*). Research Journal of Environmental and Earth Sciences 2010; 2(1):31-35.
14. Mahale SM, Goswami-Giri AS. Composition and Characterization of Refined Oil Compared with Its Crude Oil from Waste Obtained from *Mangifera indica.* Asian J. Research Chem 2011; 4(9):1415-1419.
15. Maryam F, Mohammad EB. Evaluation of Physicochemical Properties of Iranian Mango Seed Kernel Oil. 2nd International Conference on Nutrition and Food Sciences, IPCBEE 2013; 53:9.
16. Kittiphoom S. Utilization of Mango seed. International Food Research Journal 2012; 19(4):1325-1335.
17. Nilani P, Raveesha P, Kasthuribai NBRN, Duraisamy B, Dhamodaran P, Elango K *et al.* Formulation and evaluation of polysaccharide based biopolymer – an ecofriendly alternative for synthetic polymer. J Pharm Sci. & Res 2010; 2(3):178-184.
18. Ashoush IS, Gadallah. Utilization of Mango Peels and

Seed Kernels Powders as Sources of Phytochemicals in Biscuit M. G. E. World J Dairy Food Sci 2011; 6(1):35-42.
19. Elham M, Leila N, Shahrooz R, Nazaruddin R. Production of Cocoa Butter Substitute via Interesterification of Mango seed oil. 13th ASEAN Food Conference, Singapore Meeting Future Food Demands: Security & Sustainability 2013.
20. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 1964; 5:600-608.
21. Liu F, Oliveira KDV, Pera-Titus M, Pouilloux Y, Clacens J-M, Decampo F *et al*. Catalytic etherification of glycerol with short chain alcohols in the presence of Lewis acids. Green Chemistry 2013; 15:901-909.
22. Liu, Li-x, Tanguy L, Liang X-f, Sun Y, Ye H, Zeng X-x. Determination of the Total Content of Polyphenols in Kudincha by Folin-Ciocalteu Colorimetry. Journal of Tea Science 2008; 2:101-106.
23. Doat J. Les tannins dans les bois tropicaux. Revue Bois et Forêts des Tropiques 1978; n°182.
24. Gaydou EM, Bouchet P. Sterols, Methyl Sterols, Triterpene Alcohols and Fatty Acids of the Kernel Fat of Different Malagasy Mango (*Mangifera indica*) Varieties. Journal of the American oil chemists' Society 1984; 61:1589-1593.
25. Klaus F, Dieter L, Miranda GD, Marijke B, Jochem K, Silke W *et al*. Moderately elevated plant sterol levels are associated with reduced cardiovascular risk—The LASA study. Atherosclerosis 2008; 196:283-288.
26. Enaam Y, Backheet Gallotannin and flavonoid glycosides from stem bark of *Acer negundo* L. Bull Pharm Sci 2003; 26(part 1):77-82.
27. Makoto N, Takashi Y. Tannins and related compounds, part 5, isolation and characterization of polygalloylglucoses from Chinese gallotannin. J Chem Soc Perkin trans 1982; 2963-2968.
28. Bag A, Bhattacharaya SK, Chattopadhyay RR. Isolation and identification of a gallotannin 1, 2,6-tri-O-galloyl-β-D-glucopyranose from hydroalcoholic extract of *Terminalia chebula* fruits effective against multidrug-resistant uropathogens. Journal of Applied Microbiology 2013; 115:390-397.
29. Berardini N, Carle R, Schieber A. Characterization of gallotannins and benzophenone derivatives from mango (Mangifera indica L.cv." Tommy Atkins") peels, pulp and kernels by high performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 2004; 18:2208-2216.
30. Sonia G, Sáyago-Ayerdi, Cristina L, Moreno-Hernández, Efigenia Montalvo-González, Ma Lourdes García-Magaña, Miguel Mata-Montes de Oca, Joseph Lluís Torres, Jara Pérez-Jiménez Mexican "Ataulfo" mango (*Mangifera indica* L.) as a source of hydrolyzable tannins. Analysis by MALDI-TOF/TOF MS. Food Research International 2013; 51:188-194.
31. Li-x L, Tanguy L, Liang X-f, Sun Y, Ye H, Zeng X-x *et al*. Determination of the Total Content of Polyphenols in Kudincha by Folin-Ciocalteu Colorimetry. Journal of Tea Science 2008; 2:101-106.