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STANDARDISATION OF LEAVES OF *MALLOTUS PHILIPPENSIS* (LAM.) MUELL. ARG. AS PER WHO GUIDELINES

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ABSTRACT

The standardization of Leaves of *Mallotus philippensis* (Lam.) Mull. Arg. was carried out according to the quality control methods suggested by WHO. The powder material was tested for various parameters like macroscopy, microscopy, foreign matter, ash value, extractable matter, crude fibre content, moisture content, swelling index, foaming index, pesticide residues, Arsenic and heavy metals, microbial contamination etc. The preliminary phytochemical screening showed the presence of amino acids, phenolic compounds, proteins, steroids, terpenoids and carbohydrates in various extracts. The material was free from microbial contamination. The TLC profile of extracts showed the presence of saponins, flavonoids, bitter principles, acidic metabolites, cardiac glycosides and alkaloids.The HPTLC studies showed the presence of 16 numbers of compounds.

KEYWORDS: ***Mallotus philippensis*** **(Lam.) Muell. Arg., Leaves, WHO guidelines, TLC studies, HPTLC analysis**

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INTRODUCTION

In olden days, medicines were prepared for ready use from herbs. Therefore, standardization was not necessary. There is a need of standardization of herbal drugs, when they were formulated and stored. There is a great claim of the herbal drugs from the developing and also developed nations because of their efficacy, safety and lesser side effects as compared to synthetic molecules. Nearly, 80% of the world population depends upon traditional plant based – medicines. They are used for the treatment of various disorders like Asthma, Eczema, Premenstrual syndrome, Rheumatoid arthritis, Migraine, Menopausal symptoms, Chronic fatigue, Irritable bowel syndrome and Cancer, among others. The lack of standardization is the key factor why the herbals are not accepted. Hence, standardization has become an essential component of the herbal drug/products that ensures the benefits of the herbal drugs. The effectiveness and acceptability of the herbal products greatly depend upon its quality [1].WHO has developed general global guidelines to facilitate the registration and regulation of herbal medicines (Traditional medicines) by establishing the foundation for a harmonized regulatory standard to meet the common demands of the Region [2].*Mallotus philippensis* (Lam.) Muell. Arg., a dioceous plant, popularly known as a Monkey face tree, having height up to 10 meters; Leaves are alternate, variable in size, ovate or ovate lanceolate, acuminate, entire or slightly toothed, glabrous above, pubescent and with numerous orbicular red glands beneath reticulated veined, base rounded or acute, strongly 3-nerved at the base and with 4-7 pairs of nerves above the basal ones. Petioles are long, cylindrical fulvous pubescent with two small sessile glands one on each side of the summit. Flowers are dioecious, small, the male clustered sessile or very shortly pedicellate, in erect terminal spikes which are usually several together and often longer than the leaves; the female sessile are nearly so, in short spikes. Male flowers have sepals 4 (rarely 5), 3 mm long, lanceolate, acute. Stamens are numerous, bracts 15 mm long, broadly ovate, acute, buds-globosely ovoid. Female flowers have calyx divided nearly to the base, sepals 3 or 4, thicker than in the male, ovate-lanceolate. Ovary with red glands, 3-celled; styles 3 simple papillose, Capsules 8-13 mm in diameter, 3-lobed, loculicidally 3-valved, covered with a bright red powder consisting of minute stellate hairs and fine grains of a red resinous substance soluble in alcohol and ether. Fruits are dehiscent, anther 2 locular, 3-valved capsule coloured red from the numerous red glands upon it. Seeds are 4 mm in diameter, subglobose, thick, and black in colour [3]. Traditionally, Kamala powder was used as an oral contraceptive; the anti-fertility factor was identified as Rottlerin [4]. It possesses anthelmintic property. According to Ayurveda, the leaves were bitter, cooling and give appetite. Fruit was used as purgative, anthelmintic, vulnerary, detergent, maturant, carminative, alexiteric; heal ulcers and wounds, tumours, stone in the bladder; useful in bronchitis, diseases of the abdomen, and enlargement of spleen (Ayurveda) [5].The present study focused on standardization of *Mallotus philippensis* (Lam.) Muell. Arg. to reach it into the global community.

MATERIALS AND METHODS

(i)Collection of the plant material

The leaves of *Mallotus philipinensis* (Lam.) Muell. Arg. were collected from Topsengattupattu at Pachaimalai hills during Februry 2008 and authenticated by Prof. P. Jeyaraman, the Director of Plant Anatomy Research Centre (PARC), Pharmacognosy Institute, West Thambaram, Chennai – 45. The voucher specimen was deposited in the Museum of the Department of Pharmacognosy, Adhiparasakthi College of Pharmacy, Melmaruvathur, India.

(ii)Macroscopical and Microscopical evaluation [12 – 15]

The macroscopy and microscopy of the leaves were studied (Brain and Turner, 1975). The macroscopic and organoleptic behaviours such as shape, size, surface characteristics, colour, odour and taste of the leaf were observed. The specimens were

prepared for sectioning (Sass, 1940). Dewaxing of the sections was done (Johansen, 1940). The sections were stained with O toluidine blue and the characteristics were observed. (O'Brien et. al., 1964). The microscopical studies of the leaf powder were carried out. For studying the stomatal morphology, venation pattern and trichome distribution, the paradermal sections were taken. The leaf constants such as stomatal index, vein islet number, veinlet termination number and palisade ratio were studied after clearing the leaf with 5% sodium hydroxide. The different cellular components of powder were also studied and measured [16 – 17].

(iii)Physical evaluation

The leaves were dried under shade, powdered and passed through 40 meshes and stored in closed vessel for further use. The parameters such as foreign matter, total ash, acid insoluble ash, water soluble ash, sulphated ash, extractable matter by hot extraction and cold maceration methods, water and volatile matter, volatile oil, swelling index and foaming index were studied. The determinations were performed in triplicate and the results were expressed as mean ± SEM. The percentage w/w values were expressed as air dried drug basis.

(iv)Qualitative screening of phytoconstituents [18 – 19]

The moderately coarse powder was successively extracted with petroleum ether, benzene, chloroform, acetone, methanol and water. The extracts were subjected for preliminary phytochemical screening to find out the presence of different types of phytoconstituents.

(v)Quantitative evaluation of phytoconstituents [20]

The quantitative determination of phytoconstituents was also carried out to find out the amount of phytoconstituents present in the powdered drug.

(vi)Thin Layer Chromatographic studies [21]

The thin layer chromatographic studies were carried out to find out various types of phytoconstituents present in the methanolic extract of leaf.

(vii)High Performance Thin Layer Chromatographic studies [22 – 24]

The CAMAG HPTLC System equipped with Automatic TLC Sampler 4, TLC scanner 3, Reprostar 3 with DXA252 Digital camera for photo documentation, controlled by WinCATS-4 software was used for HPTLC analysis. All the solvents used for HPTLC analysis were obtained from MERCK. The 2 µL, 4 µL, 6 µL and 8 µL of samples of methanolic extract were spotted in the form of band of length 8 mm with a CAMAG Automatic TLC Sampler 4 having a microlitre syringe (size: 25 µL) on pre-coated Silica Gel glass plate 60 F 254 (10 x 10 cm). The plate was pre-washed by methanol. The sample loaded plates were kept in TLC trough developing chamber (20 x 10 cm) with the respective mobile phase as Toluene: Ethyl acetate: Formic acid: Methanol (30:30:7.5:2.5) and the plate was developed in the respective mobile phase up to 90 mm. The optimized chamber saturation time for mobile phase was 15 – 20 min at room temperature 25±2°C. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was scanned and photo-documented at 254 and 366 nm.

(viii)Determination of pesticide residues [25 – 27]

10 g of the powder was homogenized with ethyl acetate and was extracted with magnesium sulphate and sodium acetate. This was shaken vigorously for 1 minute and centrifuged for 1 minute at > 1,500 rpm to sediment the solid. The supernatant was transferred to an auto sampler vial and it was tested using TIC-MS methods.

(ix)Determination of Arsenic, Cadmium and Lead [28]

2.5 ± 0.1 g of well homogenized sample was weighed in a microwave vessel. To this, 5ml of Suprapure Nitric acid and 2ml of 30% Hydrogen peroxide were added. For every batch, the blank, duplicate and spiked samples were run. The vessels were closed and kept in the microwave digester. Initially, the vessels were kept in 100 watts power, at the temperature of 70°C for 10 minutes. Then, they were kept in 200 watts power, at the temperature of 100°C for 10 minutes. Finally, they were kept in 400 watts power, at

the temperature of 140°C for 10 minutes. They were kept for 10 minutes. After completion of the digestion process, the vessels were cooled thoroughly. The vessels were opened. The lid and walls of the container were rinsed down. The solutions were transferred separately to a 25ml volumetric flask and diluted to mark. They were taken for analysis by Atomic Absorption Spectroscopy.

Calibration range for AAS

Arsenic : 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/lit
Cadmium : 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/lit
Lead : 0.04, 0.06, 0.08, 0.10, 0.12, 0.15 mg/lit

Table 1
Preparation of standard solution

S. No.	Elements	Stock solution A (ppm)	Preparation of sec. stock B	Conc. of sec. stock B (ppm)	Preparation of working stock C	Conc. of working stock C (ppm)	Preparation of working stock D	Conc. of working stock D (ppm)
1.	Arsenic	1000	2.5 ml of A is diluted to 50 ml	50	10 ml of B is diluted to 50 ml	10	10 ml of C is diluted to 50 ml	1.0
2.	Cadmium							
3.	Lead							

The individual working standards were prepared as per the calibration range by diluting a suitable aliquot from working stock D to a volume of 50 ml. All dilutions from the stock solution were made with 3% of Nitric acid.

(x)Determination of microorganisms [6]

The presence of microorganisms such as *Escherichia coli*, *Salmonella typhimurium, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* present in the plant material was tested.

(xi)Determination of aflatoxins [28]

20 gm of the leaf powder was taken. To this, 100 ml of acetonitrile : water (60:40) was added and the mixture was swirled for 2 minutes. The solution was filtered through Whatmann filter paper No.4. The filtrate was retained. 2 ml of the filtrate was diluted with 48 ml of phosphate buffered saline (pH 7.4). The sample was diluted with 50 ml of acetonitrile and added slowly to the column. The column was washed by passing 20 ml of phosphate buffered saline at a flow rate of approximately 5 ml per minute. After washing the column, it was rapidly dried by passing the air. Then, 1.5 ml of 100 % methanol was pipetted into the glass barrel. The toxin was eluted from the column into the glass vial by passing slowly the methanol through the column at the flow rate of 1 drop per second. The elute was subjected for the analysis of HPLC-Fluorescent detector. Preparation of working standards

Reference standard stock : Mixed standards of B1, B2, G1, G2 (Supelco),
B1, G1 : 1.0 ppm
B2, G2 : 0.3 ppm

Table 2
Preparation of working standards

Stock solution - A	Secondary solution – B	Secondary solution – C
	1ml of stock A diluted to 10 ml with methanol	1ml of Standard B diluted to 10 ml with methanol
B1 1.0 ppm	100 ppb	10 ppb
B2 0.3 ppm	30 ppb	3.0 ppb
G1 1.0 ppm	100 ppb	10 ppb
G2 0.3 ppm	30 ppb	3.0 ppb

Series of working standards were prepared by diluting 10, 20, 30, 40 & 50 µl of standard C to 1ml with 1:1 methanol and water.

RESULTS

(i)Macroscopical and microscopical evaluation

Mallotus philippensis (Lam.) Muell. Arg. Leaves

Figure 1
Macroscopy of leaves of Mallotus philippensis (Lam.) Muell. Arg.

The leaves were ovate, lanceolate, 6 -18 x 2-10 cm in size, pubescent below, base acute to rounded, margin entire, or sparsely serrate, apex acute to acuminate, lateral nerves about 6 pairs; basal pair was prominent. It was pale green in colour, odourless and slightlybitter in taste.In cross sectional view of the leaf, the midrib was quite thick and prominent. The lamina was thin. The midrib was 1mm thick and 900 µm wide.

Midrib

Transverse Section of leaf

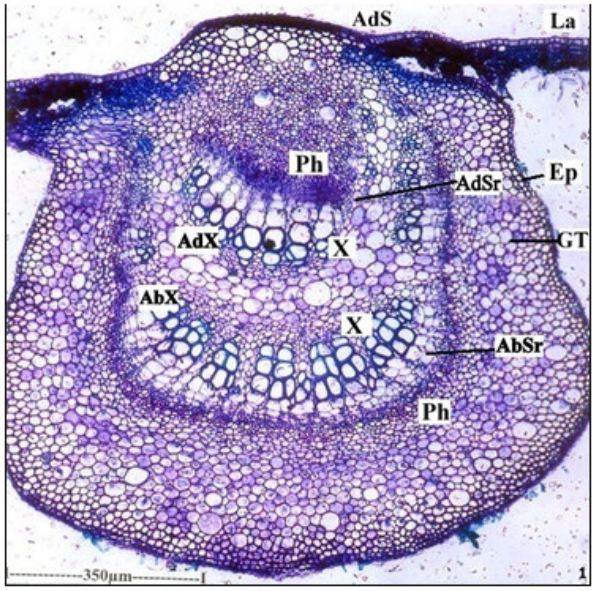

Figure 2
Transverse Section of leaf through Midrib (10X)
(AdS – Adaxial side, AbSr – Abaxial strand, AdSr - Adaxial strand, AbX – Abaxial xylem, AdX – Adaxial xylem, Ep – Epidermis, GT – Ground Tissue, La – Lamina, Ph – Phloem, X – Xylem)

It was wide and thick. It was shield shaped with more or less flat adaxial side and a dilated abaxial part (Figure 2). It had a thin less distinct epidermal layer comprising of small thick walled squarish cells. The ground tissue was wide, including outer two to four layers of thick walled small compact cells. The remaining ground tissue was wider, angular, compact and thin walled. The cells dispersed in the ground tissue were wider and had circular cavities which represent laticifers or latex secreting structures.The vascular system was two-stranded: one strand was abaxial – median in position, larger in size, too wide and deep bowl shaped. This strand had several radial segments of xylem. Each segment had two compact rows of wide, thick walled xylem elements and thin continuous layer of phloem along the lower end of the xylem. The other strand was adaxial in position with uniseriate, wide xylem and the phloem was situated along the upper part of the xylem. The xylem strands of the adaxial and abaxial vascular bundles were juxtaposed.

Lateral vein

The lateral vein was similar to the midrib. It was prominently projecting towards the abaxial side; it was 600 µm thick. It also had two vascular strands which were juxtaposed by their xylem strands.

Lamina

Transverse section of lamina

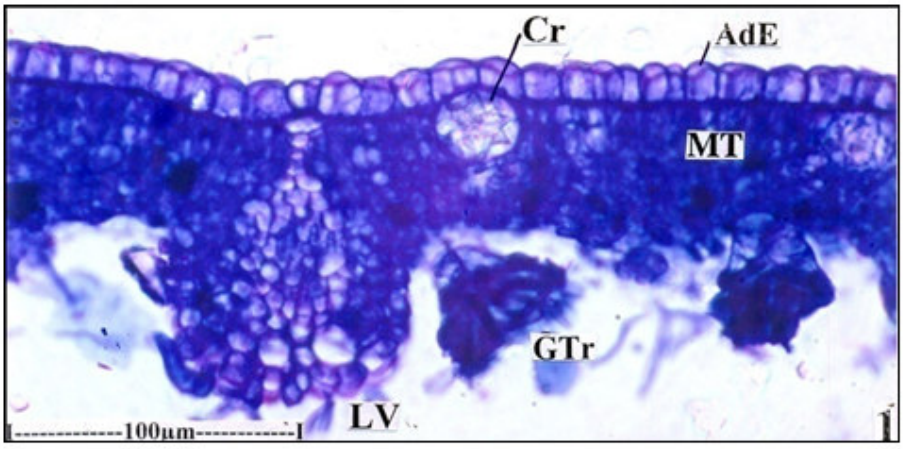

Figure 3
Transverse section of lamina showing crystals, glandular and nonglandular trichomes. (40X)
(AdE – Adaxial epidermis; Cr – Crystal; GTr. - glandular trichomes; LV – Lateral vein; MT – Mesophyll tissue)

The lamina was dorsiventral with smooth adaxial side, much wavy and densely pubescent adaxial side. The adaxial epidermis was thick, measuring 40 µm; the cells were vertically elongated with semicircular outer anticlinical walls. The mesophyll tissue had two layers of short squarish palisade cells and three layers of circular compact spongy parenchymatous cells. These were wide circular cavities in the adaxial part of the lamina in which large druses of calcium oxalate crystals were located.The epidermal trichomes were densely distributed on the abaxial side of the lamina. Glandular and non-glandular types of trichomes are seen in the epidermis. Each clustered trichomes of non-glandular type consist of a basal cell, buried in the epidermis. The basal cells bore cluster of unicellular, pointed dead trichomes. The clusters of trichomes were directed laterally, so that, the trichomes appeared “stellate” in the surface view.The second type of trichomes was glandular peltate type. The trichomes had one or two celled stalk at the apex and a spherical, darkly staining glandular head. The cells were 8-12 and radiating from central point. The glandular head was 140-180 µm in diameter.

Powder Microscopy of leaf

The leaf powder exhibited their epidermal layer of the adaxial epidermis. The epidermal cells were amoeboid in outline due to wavy anticlinal walls. The epidermis was apostomatic. Long, unicellular, non-glandular trichomes were seen arising from the coastal cells of the epidermis.

Leaf constant determination

The stomatal index (of the abaxial side), Palisade ratio, vein islet number and veinlet termination number of the leaf was 23.33, 3 – 5, 4 – 6, and 6 – 8.

Microscopical measurements

The length and width of fibres, length of trichomes and the dimension of calcium oxalate crystals were studied by using eye piece and stage micrometers, shown in Table 3.

Table 3

Microscopical measurements of leaves of Mallotus philippensis (Lam.) Muell. Arg.

S. No	Microscopical character	Dimension	Minimum (µm)	Average (µm)	Maximum (µm)
1.	Fibres	Length	150	387.5	625
		Width	12.5	31.5	50
2.	Trichomes i) non – glandular solitary trichomes ii) stellate type of trichomes	 Length Length	 400 300	 210 225	 20 150
2.	Calcium oxalate crystals	Length	26	65	104
		Breadth	26	84.5	143

(ii)Physical evaluation

The physical parameters are given in Table 4.

Table 4

Physical evaluation of leaves of *Mallotus philippensis* (Lam.) Muell. Arg.

S. No.	PHYSICAL CONSTANTS	VALUES (% w/w)
1.	Foreign Organic matter	0.0044 ± 0.00075*
2.	Ash values Total Ash Water soluble Ash Acid insoluble Ash Sulphated Ash	 4.29 ± 0.053* 1.55 ± 0.065* 0.52 ± 0.03* 2.33 ± 0.05*
3.	Extractable matter Hot extraction (Methanol) Hot extraction (Water) Cold maceration (Methanol) Cold maceration (Water)	 10.12 ± 0.23* 8.53 ± 0.25* 8.98 ± 0.90* 5.83 ± 0.26*
4.	Moisture content	3.23 ± 0.02*
5.	Volatile oil	0
6.	Crude fibre content	14.05 ± 0.58*
7.	Swelling Index	1.2 ± 0.12*
8.	Foaming Index	< 100

(iv) Qualitative screening of phytoconstituents

The following table 5 showed the nature and quantity of the extracts obtained by successive solvent extraction. The table 6 showed the phytoconstituents present in the extracts.

Table 5
Sucessive solvent extraction of leaf powder

Solvent	Colour	Consistency	Extractive value (%w/w)
Petroleum ether	Greenish brown	Sticky mass	2.08
Benzene	Olive green	Sticky mass	0.56
Chloroform	Olive green	Semi solid	1.36
Acetone	Dark green	Sticky mass	0.48
Methanol	Yellowish brown	Semi solid	10.98
Water	Dark brown	Solid	4.60

Table 6
Quantitative screening of phytoconstituents

Plant Constituents	Extracts					
	Pet. ether	Benzene	Chloroform	Acetone	Methanol	Water
Alkaloids	-	-	-	-	-	-
Amino acids	+	-	+	+	+	-
Anthraquinones	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Phenolic groups	+	+	+	+	+	+
Proteins	+	-	+	+	+	-
Gums. Resins & oils	+	+	-	-	-	-
Catechins	-	-	-	-	-	-
Quinones	-	-	-	-	-	-
Steroids	+	+	+	+	+	-
Saponins	-	-	-	-	-	+
Tannins	-	-	-	-	-	-
Terpenoids	-	-	+	+	+	+
Carbohydrates	-	-	+	+	+	+

Where'+' – presence, '-' – absence

(v) Quantitative screening of phytoconstituents

The quantitative analysis of leaf showed no measurable quantity of tannins present in the leaf powder. The presence of saponins, flavonoids and phenols were 3.5% w/w, 5.68% w/w and 0.47% respectively.

(vi) Thin layer chromatographic studies

The methanoilc extract showed the presence of 6 different types of phytoconstituents, given in table 7.

Table 7
Thin layer chromatographic studies of methanolic extract of leaves of Mallotus philippensis (Lam.) Muell. Arg.

S. No.	Phytoconstituents & Solvent system	Spot No.	Distance travelled by the solute	Distance travelled by the solvent	Detecting agents	R_f Value	Colour
1.	**Saponins** n-butanol: acetic acid: water (15:3:12)	1	0.7	6.9	Iodine solution	0.10	Pale brown
		2	1.8	"		0.26	Pale brown
		3	4.5	"		0.65	Pale brown
		4	4.9	"		0.71	Pale brown
		5	5.3	"		0.77	Pale brown
2.	**Flavonoids** Ethyl acetate: formic acid: glacial acetic acid: water (30:3.3:3.3:8.1)	1	0.8	9.5	Iodine solution	0.08	Pale yellow
		2	1.3	"		0.14	Pale yellow
		3	9.4	"		0.99	Pale yellow
	Bitter principles	1	1.2	12.0		0.1	Brown

3.	Ethyl acetate: methanol: water (23.1:3.9:1.74)	2	3.0	”	Iodine solution	0.25	Brownish orange
		3	4.5	”		0.38	Brown
		4	6.4	”		0.53	Yellow
		5	10.8	”		0.9	Yellow
4.	**Acidic metabolites** Toluene: ethyl acetate: acetic acid (24:5.4:0.6)	1	6.3	11.2	Vanillin in H_2SO_4	0.56	Brown
		2	8.9	”		0.8	Pale yellow
5.	**Cardiac glycosides** Ethyl acetate: methanol: water (24.3:3.3:2.4)	1	10.1	11.0	Vanillin in H_2SO_4	0.92	Brown
6.	**Alkaloids** Toluene: ethyl acetate: diethylamine (70:20:10)	1	1.5	11.4	Vanillin in H_2SO_4	0.13	Pale yellow
		2	2.1	”		0.18	Pale green
		3	4.7	”		0.41	Pale green
		4	6.0	”		0.53	Pale green
		5	6.5	”		0.57	Green
		6	6.9	”		0.61	Pale green
		7	7.5	”		0.66	Green

(vii) High Pressure Thin Layer Chromatographic studies

It showed the presence of 16 numbers of compounds present in the methanolic extract which was confirmed by the peaks and R_f values obtained.

Chromatogram and Photos of HPTLC profile of the methanolic extract

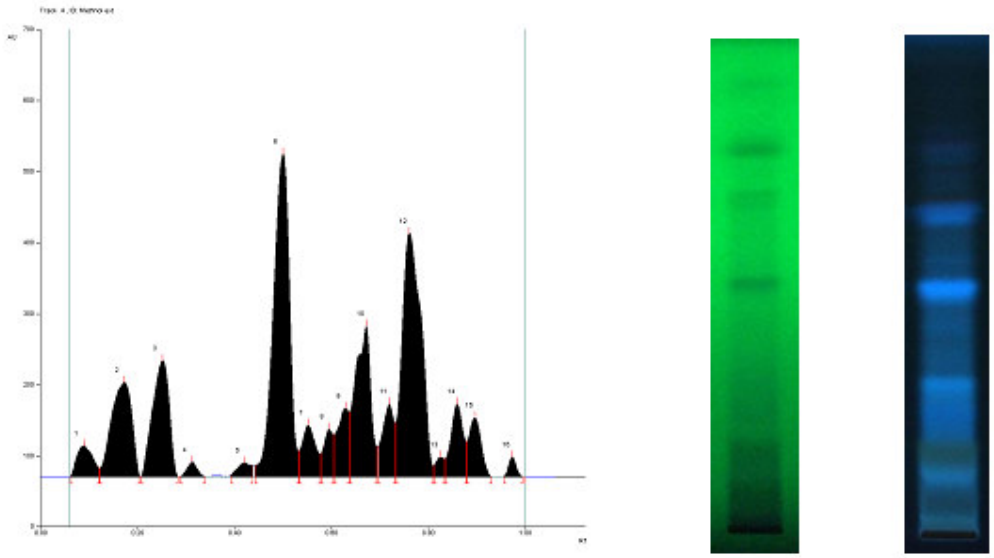

Figure 4
The chromatogram obtained with 8 µL of the methanolic extract and photos obtained by 254 nm and 366 nm of the HPTLC analysis.

Table 8
R_f values of methanolic extract of leaves obtained by HPTLC analysis

Peak	Start R_f	Start Height	Max Rf	Max Height	Max %	End R_f	End Height	Area	Area %	Assigned substance
1.	0.06	0.5	0.09	43.7	2.22	0.12	12.4	1343.8	2.32	Unknown
2.	0.12	12.5	0.17	133.0	6.75	0.20	0.6	5177.0	8.95	Unknown
3.	0.21	0.3	0.25	163.8	8.31	0.28	0.5	5202.4	8.99	Unknown
4.	0.29	0.7	0.31	20.7	1.05	0.34	0.0	404.1	0.70	Unknown
5.	0.39	2.7	0.42	20.0	1.02	0.45	15.9	523.2	0.90	Unknown
6.	0.44	15.9	0.50	454.8	23.07	0.53	36.4	13787.5	23.84	Unknown
7.	0.53	36.7	0.55	72.5	3.68	0.58	31.4	1888.3	3.26	Unknown
8.	0.58	32.3	0.60	67.2	3.41	0.60	60.1	1207.9	2.09	Unknown
9.	0.61	60.0	0.60	96.3	4.89	0.64	91.0	2013.8	3.48	Unknown
10.	0.64	91.2	0.67	212.0	10.76	0.69	42.9	6429.3	11.11	Unknown
11.	0.70	43.1	0.72	102.7	5.21	0.73	76.0	2327.9	4.02	Unknown
12.	0.73	77.0	0.76	342.8	17.39	0.81	15.3	12358.7	21.37	Unknown
13.	0.81	15.9	0.82	27.7	1.40	0.83	25.1	430.5	0.74	Unknown
14.	0.83	25.4	0.86	102.5	5.20	0.88	49.4	2480.8	4.29	Unknown
15.	0.88	49.9	0.90	83.0	4.21	0.93	0.0	1861.7	3.22	Unknown
16.	0.96	0.2	0.97	28.2	1.43	1.00	0.2	407.2	0.70	Unknown

(viii)Determination of pesticide residues, heavy metals, microorganisms and aflatoxins etc

The presence of organo chlorine and organo phosphorous were within below daily limit as 0.005 mg/kg. The each of the heavy metals such as Arsenic, Cadmium and Lead were found with the below daily limit of 0.05 mg/kg. The microorganisms such as *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were absent in the leaf powder. The 10 cells of *Candida albicans* present in per gm of the leaf powder. The quantity of Aflatoxin B1, B2, G1 and G2 present in the leaf powder were 0.293, 0.090, 0.303 and 0.090 µg/kg respectively.

DISCUSSION

The leaves were ovate, lanceolate in shape and varied in sizes. The dorsiventral leaf showed calcium oxalate crystals in the lamina. Glandular and non-glandular types of trichomes were seen in the epidermis. An Abundent paracytic type of stomatas was seen in the abaxial side of the epidermis. The size of the fibres present in the leaf powder was 150 – 387.5 – 625 µm. The foreign organic matter present in the leaf was minimum as 0.0044% w/w and it was within the limit. The ash value of 4.29%w/w showed the presence of inorganic composition and the sulphated ash of 2.33% w/w was the total carbonates of the leaf. The 10.12% w/w of extractable matter of methanol by hot extraction gave the choice of solvent and the method of extraction. Though, the moisture content of 3.23% w/w of leaf had no identification of microbial contamination. The foaming index of less than 100 showed the presence of less quantity of saponins in the leaf powder. There was no volatile matter in the powder. The presence of phenolic compounds and steroids showed such compounds present in various extracts and they were further identified by TLC. The HPTLC studies showed the presence of 16 numbers of compounds, which would be extracted with methanol and further fractionated by different types of solvents. The fraction would be used for the determinations of pharmacological activities.

CONCLUSION

The standardization of leaf material of *Mallotus philippensis* (Lam.) Muell. Arg. was done as per WHO guidelines. This work satisfies the aim of reaching the plant and its uses to the global community to the maximum extent.

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