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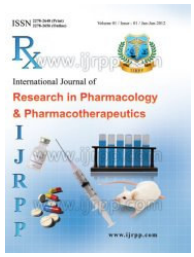

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Pharmacognostical, phytochemical, physio-chemical and acute toxicity studieson *Morinda reticulate Gamble*

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ABSTRACT

The present research work was expanded to investigate the pharmacognostic properties, physiochemical constants, phytochemical tests for the existence of different secondary metabolites and its toxicity was evaluated using the model of acute toxicity analysis for the areal sections of Morindareticulata Gamble. Photomicrograph experiments have been conducted in pharmacognostic work. Physiochemical constants such as ash value, drying loss and extractive values were evaluated for successive solvents with an increasing order of polarity. With various solvent extracts, preliminary phytochemical screening was performed, revealing the presence of carbohydrates, proteins ,aminoacids, flavonoids, terpenoids, tannins and phytosterols. It was found that there were absent alkaloids, glycosides, saponins, liquid oil, fixed oil, and gums and mucilage. The original acute toxicological analysis found that the root methanol extract was healthy and non-toxic.

Keywords: *Morinda reticulate Gamble*, Microscopical, Physiochemical, Acutetoxicological studies.

INTRODUCTION

Conventionally, herbal medicines are used to prevent/cure diseases/meloriate well being globally, thus drawing more exposure to it. In more multidirectional trials, including new drug creation, clinical documentation of herbal treatments has shown to be helpful. In resolving health care issues, herbal plants and conventional health care programs are increasingly attracting popularity. As an intrinsic part of their society, the majority of developed countries have adopted western medical practise.[1-5]

Morinda reticulate belongs to the family of Rubiaceae, It is large woody climbing shrubs. Leaves 6-12 x 2-4.5 cm, oblanceolate to linear-lanceolate, attenuate at base, caudate acuminate atapex, waxy shining above,lateralnerves10-12 pairs; petioles to 6mm long; stipulesacute, connate. Flowers white in terminal umbellate heads; peduncle 1-2 cm. Calyx truncate, limb forming a ring. Corollarotate; tubec. 1.5 mm long, very hairy within; lobes 4, oblong, recurred. Stamens 4, included. Stigma 2-fid. Syncarpium irregularly lobed, 0.5-1 cm diam., with prominent scars of calyx ring,orange; pyrenes many,

bony, pyriform, triquetrous in viscous pulp.Habit: Climber, Flowering &Fruiting: March-September, It was found in Choodal, Kallar, Kulathupuzha, Kottayali, on way to Nilamel, Boneccord, Kottur R.F., Merchiston, Thenmalai, Bonaccord, Karamanayar region [6]. Intraditional Japanese, Korean and Chinese medicine, Morinda citrifolia is considered to be an herb with biological properties, although there is no confirmed evidence of clinical efficacy.

MATERIALSANDMETHODS

Collection and authentification of plant materials

The plant *Morinda reticulatae Gamble* hook collected from western ghat, Madurai, Trichy District. Plant collections were done, during the month November 2018. The plant was authentified by Dr.P.Jayaraman, professor, PARC, West Thambaram, Chennai. The voucher specimen of the plant was deposited at the college for further reference.

Photomicrographs

Micrographs were combined with microscopic representations of tissues where possible. Photographs with varying magnifications were taken with the microscopic unit of Nikonlab picture. Bright field was used for normal observation. Polarized light was used for the study of crystals, starch grains and lignified cells. Provided that these materials have brief ringent properties, they look white again stadark back drop under polarized illumination. Scale-bars reflect the magnificence of the figures. As given in the standard Anatomy books, descriptive terms of the anatomical features are[7].

Physico-chemical constants

Determination of ash values: [8-11]

Ash value is a criterion of judging the identity or purity of crude drugs. The objective of ash vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in ananalytical determination. On incineration, crude drugs normally leave an ash usually consisting orcarbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a cruded rugreflects the care take ninits prevention. A higher limit of acidin soluble ash is imposed especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some analysis flavour mixing of sulphuric acid with the powdered crude drug before ash and this sulphated ash is normally less fusible than ordinary ash.

Ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash were determined according to Indian pharmacopoeia. For determination of different ash values, these lected plant materials were powdered and the powder was passed throughsieveno.40 and the ash values were determined using the following procedure. The values vary within fairly wide limits and are therefore an important parameter for the purpose of evaluation of crude drugs.

Determination of Total Ash

Ashing involves an oxidation of the components of the product. A high ash value is indicative of contamination, substitution or adulteration. About 3g each of powdered materials was accurately weighed and taken separately in a silica crucible, which was previously ignited and weighed. The powder was spread as a fine, even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air dried drug.

Acid in soluble ash

Ash insoluble in HCL is theresi due obtaining after extracting the sulphated ortotalash with HCL. This acid insoluble ash value particularly contamination with siliceous materials like earth ors and. The ash obtained as described above ass boiled with 25ml of 2N HCL for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug

Water soluble Ash

Water soluble ash is the part of the total ash content which soluble in water. It is a good indicator of either previous extraction of water soluble alts in the drug or in correct preparation. The ash obtained as described in the determination of total ash was boiled for 5 minutes with 25ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible ignited for 15 minutes and weighed. The procedure was repeated to get constant weight. The difference of weight was considered as water soluble ash. The percentage of the water soluble ash was calculated with reference to the air dried drugs.

Determination of sulphate dash

While determining the total ash, very high temperature (<600 C) mainly resulted in the conversion of carbon test oxides. The treatment with sulphuric acid results in sulphated ash where the oxides are converts to sulphates. [ray.b. 2008] A silica crucible was heated to redness for 10 minutes and was allowed to cool in a desiccators and weighed. About 1g of substance was accurately weighed and transferred into crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1ml concentrated sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800 +/- 25 C from until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, and a few drops of concentrated sulphuric acid were added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5mg.

Determination of extractive values

Extractive values crude drug are useful for their evaluation, especially when the constituent of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of constituents present in a crude drug. Extractive values such as alcohol soluble and water soluble extractive values were determined according to Indian pharmacopoeia. The plant materials were powdered and the powder was passed throughsieve no.20 and the extractive values were determined using the following procedures.

Alcohol soluble extractive values

About 5g each of coarsely powdered material was accurately weighed and macerated with100 ml of alcohol of specific strength, separately in a closed flask for 24 hours. Shaking was done frequently during the first 6 hours and then allowed to stand for 24 hours. This was filtered rapidly taking precaution against loss of alcohol. Then, 25ml of each of these alcoholic extracts were evaporated to dryness in a tarred flat bottom shallow dish and were dried at 105 C and weighed. Percentage of alcohol soluble extractive was calculated with reference to theair-dried drug.

Water soluble extractive value

About 5g each of coarsely powdered material was accurately weighed and macerated with100 ml of chloroform water, separately in a closed flask for 24 hours. Shaking was done frequently during the first 6 hours and then allowed to stand for 24 hours. This was filtered and then, 25 ml each of these chloroform water extracts were evaporated to dryness in a tarred flat bottom shallow dish and were dried at 105 C and weighed. Percentage of alcohol soluble extractive was calculated with reference to the air dried drug.

Loss on drying

Loss of drying is the loss in weight in percentage w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water)that can be driven under the condition specified (Desiccators or hot air oven). If the sample is the form of large crystals, then reduce the size by quick crushing to a powder.

About 1.5gm of powdered drug weighed accurately in a tarred porcelain dish which was previously dried at 105C in hot air oven of constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

Phytochemical analysis

Preliminary phytochemical investigations for secondary metabolites were conducted on different extracts obtained from bark of *Morinda reticulata*

Gamble and examined for metabolites like carbohydrates, alkaloids, glycosides, tannins, protein and aminoacid, saponins

Determination of ld 50 value

Acute oral toxicity study[12]

The procedure was followed by using OECD guidelines 423 (Acute toxic class method).

Animals

Male Swiss albino mice weighing 25-30g were used in the present study. All rats were keptat room temperature of 22-25°C in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. All animal procedures were performed after approval from the institutional ethics committee.

Procedure

Twelve animals Albino mice, (25-30gm) were selected for studies. The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable at a based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000 mg / kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized system (GHS) for the classification of chemical which cause acute toxicity.

Most of the crude extracts possess LD50 value more than 2000mg./kg of the body weight of animal used. Dose volume was administered 0.1ml/100 gm body weight to the animal by oral route. After giving the dose the toxic signs were observed within 3-4 hours.

Bodyweightofanimalsbeforeandafteradministration,onsetoftoxicityandsignsoftoxicitylikechangesinskinandfur,eyes,andmucousmembraneandalsorespiratory, circulatory,autonomicand central nervous systems and somatomotor activity and behavior pattern, signs of tremors,convulsion, salivation, diarrhoea, lethargy, sleep and coma was also to be noted, if any , wasobserved.

STATISTIC ALANALYSIS

Allthevaluesestimationswereexpressedasmean±standarderrorofmean(S.E.M)andwasanalyzedforsignificanceby ANOVAandgroupswerecomparedby Tukey-Kramermultiplecomparisontest,usingInStatv.2.02software(GraphPadSoftwareInc.).Differencesbetweengroups(pValue) wereconsidered significantat P <0.05 level.

RESULT AND DISCUSSION

Microscopical studies

Vascular cylinder

The vascular cylinder is circular and closed lay a week layer of endodermis. The cells of the endodermis are squarish. Very thick walled and liquefied. At frequent interval of the endodermis, these this walled parenchyma cells called passage cells. The passage cells are cells for transverse conduction of water. All along the winer boundary of the endodermis occur several radical strands of xylem alternating with the phloem consists of short radical law of three or four circular xylem elements; phloem occurs xylem elements; phloem occurs parenchy matous tissue of consists of polygonal, this walled compact parenchy matous cells. Alternating with the xylem in small cluster. The xylem is small clustur. The xylem and phloem strands are eushealted by sclerenchyma cells. The central part of the root is occupied by wide pith. The pith cells are circular, fairly thick walled and compact.

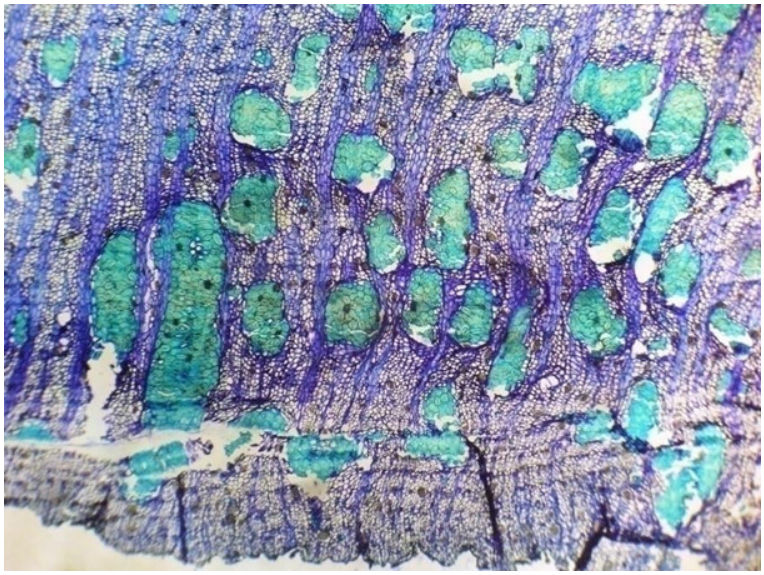

Figure 1: Microscopic characters of *Morinda reticulate* Gamblebark

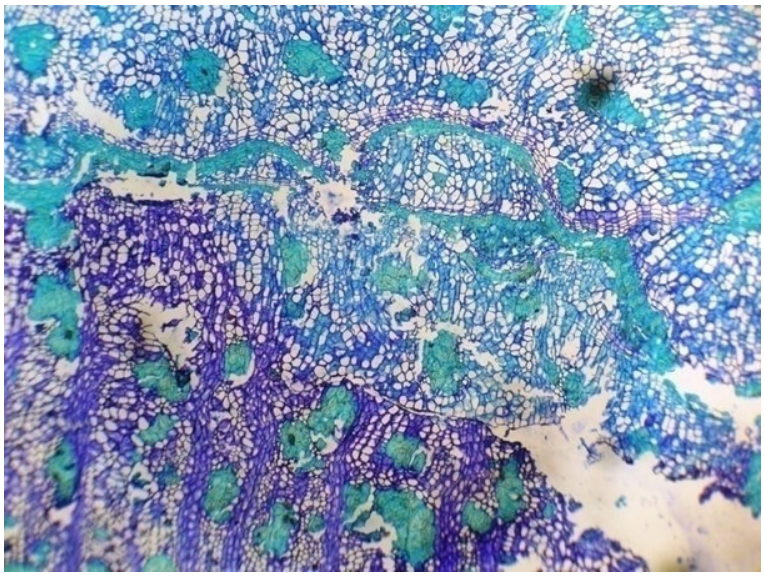

Figure 2: Transverse section of leaf showing the presence of palisade and spongymesophy ll (100X).

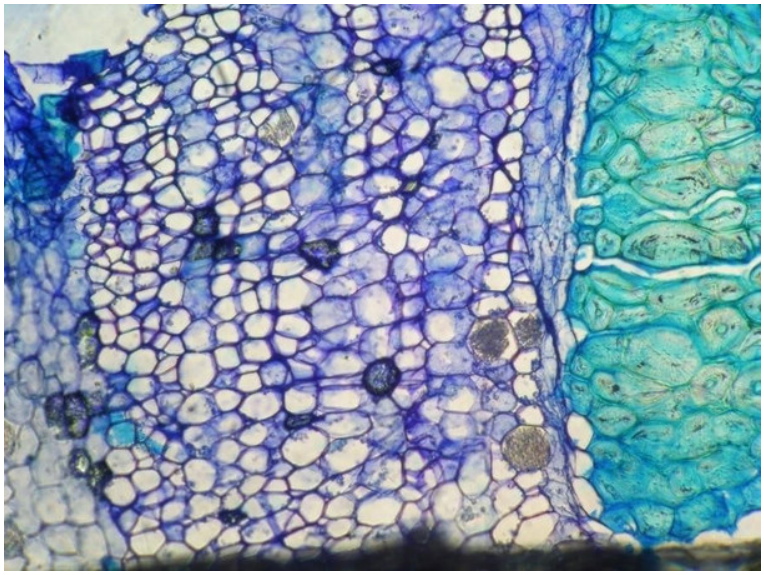

Figure 3: A microscopic observation of root section showing lignified protoxylem and phloem (100X).

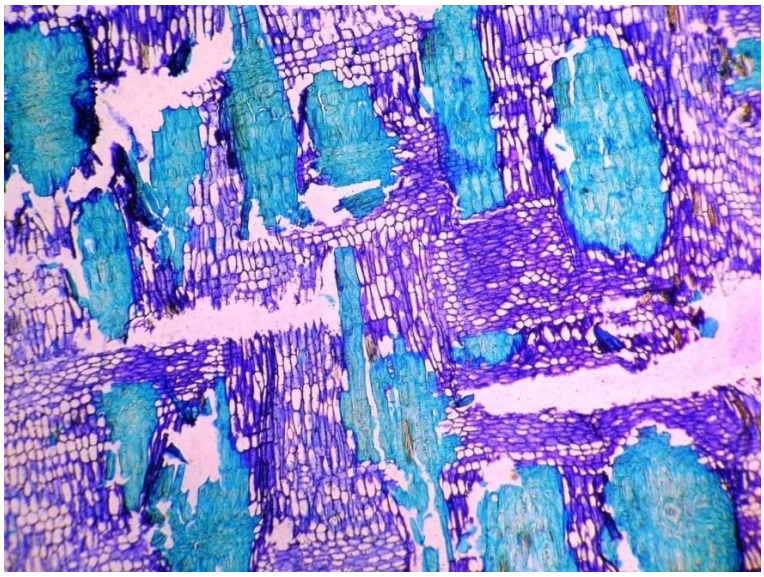

Figure 4: A piece showing the epidermal and cortical cells (10X).

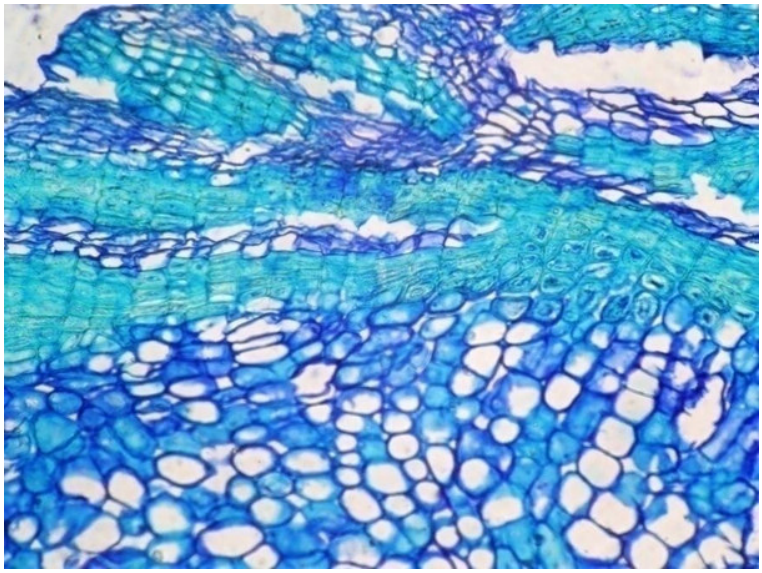

Figure 5: A microscopic observation of stem section showing epidermis and pith (100X).

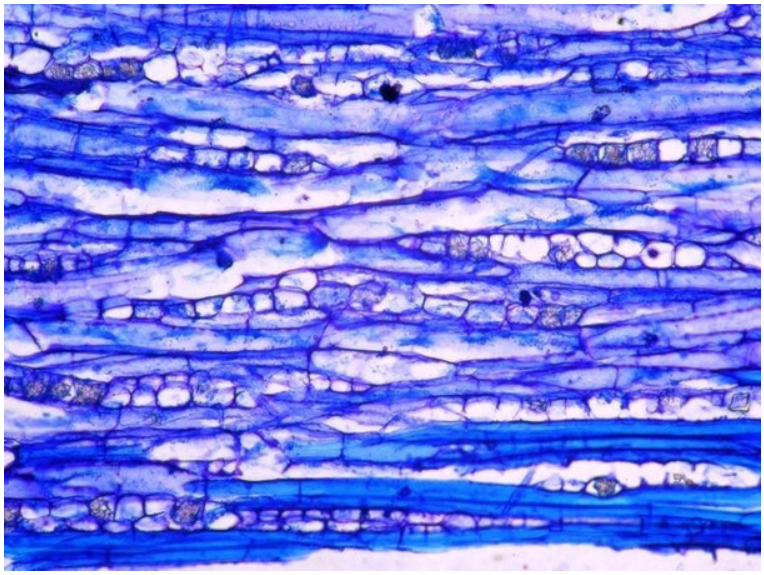

Figure 6: Transverse section of bark100 µm

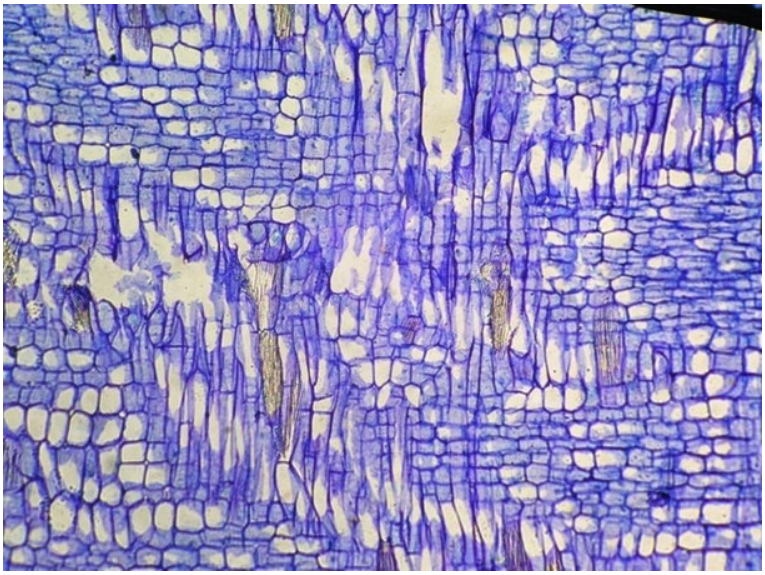

Figure 7: Transverse section of stem.100µm.

Physico-chemical constants

Determination of ash values

The total ash is 6.04 % W/W, Acid-soluble Ashis 1.01 % W/W, Acid-insoluble Ash 2.0 % W/W, Sulphated ash 1.0% W/W, Shown in table 1.

Table 1: Various Ash values of crude drug

S.No	Particulars	(%w/w)n=3
1	Total Ash	6.04
2	Acid-Insoluble Ash	1.01
3	Water soluble Ash	2.0
4	Sulphated Ash	1.0

Determination of extractive values

The alcohol soluble extractive value of the plant *Morinda reticulate Gamble* was found to be 5.7% W/W and Water soluble extractive value of *Morinda reticulata Gamble* was found to be3.1%W/W (Table 2)

Table 2: The alcohol &Water soluble extractive value

S.No	Solvent	%W/W
1.	Alcohol soluble extractive	5.7
2.	Water soluble extractive	3.1

Loss on drying

The loss on drying for root of *Morinda reticulata Gamble* gives 2.05% w/w (Table:3).

Table 3: Loss on drying

S.No	Solvent	%W/W
1.	*Morinda reticulate Gamble* powder drug	2.05

Preliminary phytochemical studies

The preliminary phytochemical studies for powder of *Morinda reticulate Gamble* extracts showed Alkaloids, Saponins, Glycosides, Carbohydrate, Flavonoids, Sterols, Proteins and amino acids, Terpenoids, Fats. The constituents present in whole plant extracts of *Morinda reticulata Gamble* was given in the table4.

Table 4: Qualitative phytochemical analysis of *Morinda reticulata*

S. No	Secondary Metabolites	Hexane	Pet. Ether	Ethyl Acetate	Methanol
1.	Alkaloids	+	-	+	+
2.	Saponin	-	-	-	+
3.	Glycosides	-	-	-	+
4.	carbohydrates	+	+	+	+
5.	Phenolic compounds &tannins	+	-	+	+
6.	Flavanoids	+	-	+	+
7.	Steroids	-	-	+	+
8.	Protien and amino acids	+	-	+	+
9.	Cums and mucilage	-	-	-	-

(+):Positive,(-):Negative

Acuteoraltoxicity studies

The acute oral toxicity of the MEMR was carried out as per OECD 423 – guidelines (Acute toxic class method). The acute toxicity studies revealed that LD50 > 2000mg/kg for theextract. Hence, the biological dose was fixed at 3 levels for *in vitro* experiments 250 µg /ml,500 µg /ml and 1000 µg /ml of MEMR and 250 and 500 mg/kg of body weight for the *in vivo* anti cancer activity, 10 mg/ml, 20 mg/ml, 30 mg/ml, and 100 -500 µg/ml for *in vitro* coagulant activity.

CONCLUSION

Pharmacognostic tests provide qualifications for the right botanical identity of the her bused for research work. With the exception of minor differences in leaf size, *Morinda reticulata Gamble* did not show any morphological changes. Micro morphological characters present then or malroot characters along with circular aperature cyclocytics to mata, alateral vein venation architecture with rectangular (or) polygonal vein islets. Inclusion of cells involves laticiferous cells, crystals of calcium oxalate and drushes. The existence of carbohydrates, proteins, aminoacids, flavonoids, terpenoids, tannins and phytosterolsis shown by preliminary phytochemical sampling utilising separate extracts. It was found that there were absent alkaloids, glycosides, saponins, liquidoil, fixed oil, and Gums and mucilage.The original acute toxicological analysis found that the root methanol extract was healthy and non-toxic.

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