ISSN No:-2456-2165

Phytochemical Screening, Physicochemical and Antioxidant Activity, TLC & Finger Print of HPTLC, Morus Alba Ethanol Extraction

Meraj Ali*, Bindu Rathaur, Urmila Nishad
Department of Pharmacy, Gaya Prasad Institute of Human Excellence, Malihabad, Lucknow *
S.B.S. Dadduji College of Pharmacy Fetehgarh, Farrukhabad.

Abstract:- The present study is design to evaluate preliminary phytochemical constituents, physiochemical properties evaluation, TLC & HPTLC fingerprinting analysis of *Morus alba Linn* (White mulberry) Leaves powder extraction.

Total phenol content, total flavonoid content, Total tannin content, TLC, HPTLC fingerprinting analysis for compounds responsible for antioxidant activity.

Phytochemical screening shows the presence of constituents like alkaloids, glycosides, carbohydrates & phytosterols, saponin, phenolic compounds, tannins, proteins, amino acids, gums and mucilage. Physicochemical studies shows that the powder was contain a moisture content 12.5% and ash value 7.5%. Total phenol content was 3.44 mg gallic acid equivalent/g, total flavonoid content was 5.00 mg rutin equivalent/g and total tannin content was 3.00 mg tannic acid equivalent/g. HPTLC fingerprinting also proofs the presence of phenol & flavonoid compounds in the leaf powder.

The results in the paper shows that the *Morus alba Linn* (White mulberry) leaf powder extract is an natural antioxidant and can be used for treatment of various disorders.

I. INTRODUCTION

Natural plants are used as very good source of nutrition persistent food as well as source of various chemical constituents operative in curing various diseases which may demand as the biologically active constituents. At the present natural plants are very much in petition in the form of drugs because of their fewer side effects, they are considered the potential resources of various bioactive compounds and are also easily available from the natural sources. In the same context *Morus alba*, the Mulberry plant which is basically famous for sericulture, the fabrication of silk done through the silkworm and the leaves are also used to diminish the symptoms of diabetes in vernacular medicine as well as for improving cardio-metabolic risks, including antihyperglycaemic, antihyperlipidaemic, antiobesity, antihypertensive, antioxidative, anti-inflammatory, anti-atherosclerotic and cardioprotective effects[1] in Chinese medicine used to treat constipation, to tonify the blood, prematurely grey hair, cough, edema, to promote urination, fever, headache, dry & sore eyes[2] and so many more, So, the leaves is used further in this study to explore some more about the biological activity of leaves.

Mulberry plant belongs to genus *Morus*having 68 species which are unisex flowering plants belonging to family Moraceae of the Utricles subclass. The plant is a shrub or tree (20 to 30 feet high) often the size of a small apple tree, having leaves which are thin, glossy, and light green in color with 5 lobes or has one lobe, two lobes, three lobes, or no lobes at all. *Morus Alba* L is also known as White Mulberry and can be grown from seed as well as planted from large cuttings of root readily. Commonly, the plantation is upraised in a block foundation with arrangement of 6 feet x 6 feet, or 8 feet x 8 feet, as plant to plant and row to row spaces. The plants are generally trimmed once a year during the monsoon season (July – August) to a height of 5–6 feet and allowed to grow with a maximum of 8–10 shoots at the top.[3] The plant is widely distributed in India, China, Japan, North Africa, Arabia, South Europe, etc.

Morus alba L. leaves had been used as substantial source of medicine, drink, and functional foods in many countries. It is used in drinks as green tea with several other herbal drugs like tulsi and aswagandha because of its immune boosting antioxidants like Chlorogenic acid, rutin, isoquercitrin, and astragalin. Anticancerous alkaloids like 1-deoxynojirimycin, morroles B–F[4], (2R,3R,4R)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-N-propionamide from the root bark and 4-O-R-D-galactopyranosyl-calystegine B2 and 3β,6β-dihydroxynortropane from the fruits[5], mulbaines A, B & C[6]. Eighteen important amino acids with calcium, potassium, sodium, magnesium, zinc, iron, copper, manganese, chromium, selenium, arsenic, vitamins and it's no caffeine property. Other chemical constituents present in leaves are coumarins, flavonoids, anthocyanins and polyphenolsincluding quercetin 3-(-malonylglucoside), rutin, isoquercitin, cyaniding-3-rutinoside apigenin, luteolin, quercetin, morin, caffeic acid, gallic acid, umbelliferone, chlorogenic acid, and kaempferol.[7]The plant extract rich in polyphenols used as a non-toxic natural healing agent, which also have high prospectiveapplications as skin-whitening agents due to its potent tyrosinase inhibitor property.[8]

ISSN No:-2456-2165

Fig 1-Morus Alba Linn plant.

➢ *Classification of Morus alba Linn*

Kingdom	Plantae – Plant
Subkingdom	Tracheobionta-Vascular Plant
Super division	Spermatophyta
Division	Magnoliophyta – Flowering Plant
Class	Magnolipsida – Dictyledons
Subclass	Hamamelididae
Order	Urticales
Family	*Moraceae*
Genus	*Morus L.*(Mulberry)
Species	*Morus alba, Morus nigra, Morus rubra,Morus indica, Morus serrate,Morus mongolica.*[6]

➢ *Same species of Moraceae family with their pharmacological uses:*

S.No.	Plants Species	Parts	Uses
1	*Morus mesozygia*	Leaves and fruits	Arthritis , Wound healing, Antioxidants and Antimicrobial compounds.
2	*Morus nigra*	Fruits	Anti-diabetic, Anti-oxidative, Anti-hyperlipidemia, Anti-inflammatory.
3	*Morus indica*	Root	Anti-inflammatory activities.
4	*Morus alba L.*	Fruit	Antioxident, Neuroprotective, Antitumor, Immunomodulative activities.[7]

➢ *Vernacular names-*

English	White mulberry
Hindi	Sahtoot, Shahtoot, Shahtut, Shehtoot, Swa, Toot, Toota, Tooth, Tul, Tulklu, Tunt, Tut, Tut.
Sanskrit	Tooda, Toola, Tudah, Tula, Tutam.
Tamil	Kamblichedi, Kampalicceti1, Kampilippucciccеti, Musukotta, Pattuppucci, Pattuppuccimaram.
Kannada	Bilee hippenerale, Bili uppu naerale, Hippal verali, Hippali naerala, Hippu naerale, Hippunerale, Kambali gida, Korigida, Tuti, Uppunute.[8]

ISSN No:-2456-2165

➢ *Description of Plant*

Morus alba Linn (white mulberry) plant has medium leaves (8-15 cm.), edges or margins. The leaves are simple, alternate and shallowly to deeply lobed. The flowers will be white & lighte yellow and the fruit is white and sweet taste. Seeds are very small in size & medicinal used. The white mulberry plant has been grown a normal temperate. The mulberry cultivate the fruit in April month. The stem is browne color and stem used in a medicinal and the family of *Moraceae*.[9]

Fig 2- Leaf veins and venation patterns reticulate, smaller veins lording and network.

➢ *Macroscopic Characteristics-*

Leaves		**Fruit**	
Colour	pale green	Colour	white
Order	characteristics	Order	sweetness
Size	8-15 c.m.	Size	2-4 c.m.
Taste	acrid	Taste	sweet[10]

➢ *Chemical Constituents of Morus alba Linn-*

Morus alba Linn. (white mulberry plants) is medicinal plant it contains following chemical constituents :(leaves, routs, frites, flower, stem.) Albino, Chalcomoracin, Moracin-c, Morin, Mulbarrofurana Q, Butain, Marrola A, 2-formyl-1H-pyrrole-1-butanol acid, Gallic acid, Tannic acid, Chlarogenic acid, Benzoic acid, Rutin astragalin, beta-sitosteral-3-O-beta-D-gluoside, beta-sitosterol, Salvigenin, Cirisimaritin, Quereetin.[7,10]

➢ *Bioactive Substances Occurring in White Mulberry*

S.No.	**Plant parts**	**Substances**	**Active compounds**
1	Leaves	Fatty acids	Palmitic acid, Linoleic acid, Eicosanoids, Oleic acid,
		Phenolic compounds	Kaempferol
2	Fruits	Phenolic compounds	Quercetin, Morin, Chlorogenic acid, Hydroxybenzoic acid, Ferulic Acid, Rutin,
		Fatty acids	Palmitic acid, Oleic acid, Linoleic acid
		Glucosides	Rutinoside cyanine, cyanine glucoside
3	Stem bark	Phenolic compounds	Maklurein, Rutin, Isoquercetin, Resveratrol, Morin, Apigenin,
		Triterpenoids	Moruslupenoic acid A, Moruslupenoic acid B, Moruslanosteryl acetate
4	Root bark	Mulberry flavonoids	Sanggenols, Kwanon, Mulberrofuran
		Phenolic compounds	Luteolin gallic acid, Sinapic acid, Resveratrol
		Lectins	Albianol [11]

II. MATERIAL METHOD-

Table: Morphological Study

S .No	Parameters	Characteristics features
1	Colour	Yellowish
2	Order	Characteristics
3	Taste	Sweet
4	Nature	Coarse powder

Fig 3: Morus Alba Fruits

Fig 4: Barks

Fig 5: Leaves powder

Fig 6: Leaves

- **Soxhlet Extraction Procedures**

Morus alba leaves was collected in Aryakul campus then fresh leave wash and dry at room temperature. All leaves dry (after two weeks) & crushed with the help of in Mortars & Pestles. The powdered material used for extracted by Soxhlet apparatus using

ISSN No:-2456-2165

the solvents for Petroleum ether, Chloroform, Acetone, Ethanol and Methanol successively. The extraction process a (40-60^{0}C) temperature for 6 hours. The concentrated product was collected and stored in refrigerator for further experimental analysis.[16,17,18]

- **Preliminary Phytochemical Screening**

The Petroleum ether, Chloroform, Acetone, Ethanol and Methanol extracts of *Morus alba Linn.* (White mulberry) will be screened for following phytocontituents class. The method for the preliminary phytochemical screening was carried away as per, Pharmacognocy.[10]

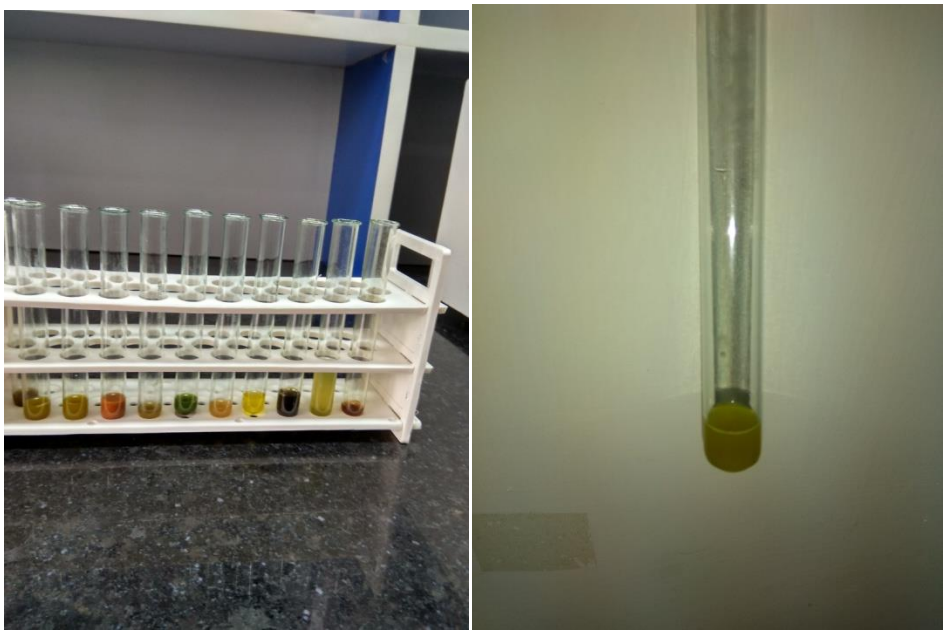

Fig 7: Chemical test

- *Thin Layer Chromatography-*

The TLC plates were prepared by using silica gel G, and were left for air drying. These Plates were activated by hot air drying in hot air oven at (80-100^{0}C) for 1 hr. Extracts from different solvents was spotted on the TLC plates. The plates were dried and developed in suitable solvents for rapid screening. The plates was run in the following solvent system and dried at room temperature (25^{0}C). The detection of TLC plate was done by Iodine chamber and UV chamber[21]. R_f value of different spots available is calculated by using the formula-

R_f value = Distance travelled by the solute / Distance travelled by the solvent

ISSN No:-2456-2165

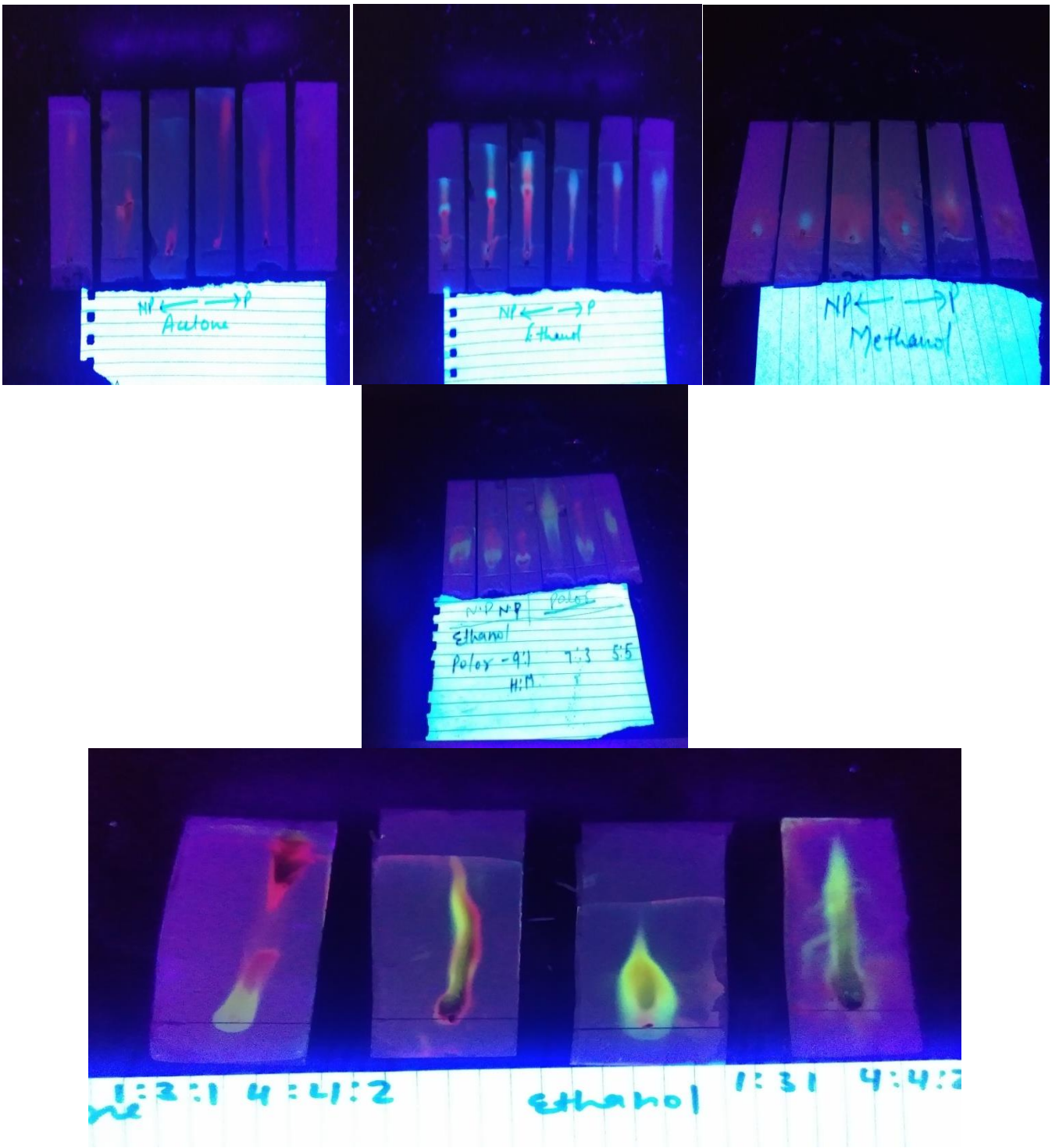

Fig 8: Visible under the U.V. chamber
Detector: UV Chamber

ISSN No:-2456-2165

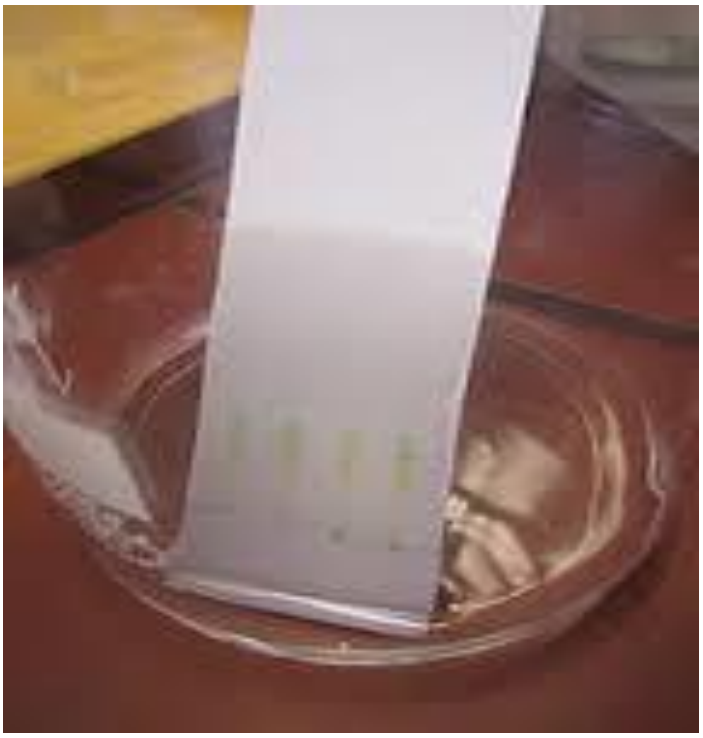

Fig. 9:- TLC performance in Laboratory

➢ *Development of HPTLC Method for Analysis of Flavonoids*

HPTLC analysis was carried out using Camag HPTLC system equipped with Linomat-v applicator and 100 ul syringes. The samples were spotted in the form of bands using micro liter syring on pre-coated silica gel 60 F254 HPTLC plates and development of the applies plate was carried out in pre-saturated Camag twin-trough chamber[25]. The developed plates were dried and analysed at 254nm and 366nm. The mobile phased used flavonoids is ethyl acetate: acetic acid: Formic acid: Water (10:1.1:1.1:2.3)[11]

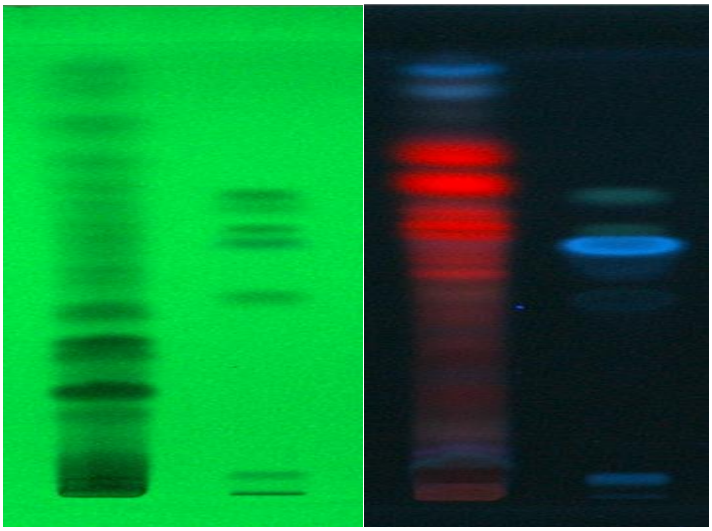

Fig. 10:- High - performance thin layer chromatography plate

III. ANTIOXIDENT CONTENT

A-Estimation of Total Phenolic Content

Phenolics were determined using gallic acid as standard. A 100µg/ml stock solution of gallic acid was prepared. From the above stock a 0.5ml aliquot was pipette out into 25ml volumetric flask. 10ml distilled water and 1.5ml Folin ciocalteus reagent was added. Then after 10 min 4 ml 20% sodium carbonate was added and volume is makeup up to 25ml using distilled water.[22]

A stock solution of 1mg/ml in methanol of ethanolic extract was prepared. From the above stock 0.5ml of extract was taken in 25ml volumetric flask. 10ml distilled water and 1.5ml Folin ciocalteus reagent was added. Then after 10 min 4 ml 20% sodium carbonate was added and volume is makeup up to 25ml using distilled water.

After 30min the absorbance of both test and standard solution was taken at 765nm. Percentage of total phenolics was calculated using the equation based on calibration curve of gallic acid:

$Y = 0.198x-0,031$
$R^2 = 0.994$

Chemical	Standard	Standard	Standard	Standard	Standard	B	Ethanol sample	Methanol sample
	1	2	3	4	5	6	7	8
Gallic acid (10%)	0.2ml	0.4ml	0.6ml	0.8ml	1ml	----	0.5ml	0.5ml
Distilled water	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml
Folin	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml
Na2Co3	4ml	4ml	4ml	4ml	4ml	4ml	4ml	4ml

25ml make up distilled water

B-Estimation of Total Flavonoid Content

Flavonoids were determined using rutin as standard. A 100µg/ml stock solution of rutin was prepared. From the above stock a 0.5ml aliquot was pipette out. 0.5ml 2% metha) methanolic aluminium chloride, few drops of distilled water and 4ml methanol was added.

A stock solution of 1mg/ml in methanol of ethanolic extract was prepared. From the above stock 0.5ml of extract was taken, add 0.5ml 2% ethanolic aluminium chloride, few drops of distilled water and 4ml methanol was added.

After 20min the absorbance of both standard and test solution was taken at 420nm. Percentage of total flavonoids was calculated using the equation based on calibration curve of rutin.[23]

$Y = 0.016x -0.204$
$R^2 = 0.926$

Chemical	Standard	Standard	Standard	Standard	Standard	B	Ethanol sample	Methanol sample
	1	2	3	4	5	6	7	8
Rutin	0.2ml	0.4ml	0.6ml	0.8ml	1.0ml	----	0.5ml	0.5ml
Alcl3	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Methanol	4.3ml	4.3ml	4.3ml	4.3ml	4.3ml	4.3ml	4.3ml	4.3ml

10ml make up methanol

C-Estimation of Total Tannin Content

Tannins were determined using tannic acid as standard. A 100µg/ml stock solution of tannic acid was prepared. From the above stock a 1ml aliquot was pipette out in 100ml volumetric flask, 5ml Folin ciocalteus reagent, few ml distilled water, 10ml saturated sodium carbonate solution were added and volume was make up by distilled water up to 100ml.

2gm powdered material were extracted and 1ml aliquot was pipette out in 100ml volumetric flask, 5ml Folin ciocalteus reagent, few ml distilled water, 10ml saturated sodium carbonate solution were added and volume was make up by distilled water up to 100ml.

Absorbance of both standard and test solution was taken at 760nm. Percentage of total tannin was calculated using the equation based on calibration curve of tannic acid.[24]

$Y = 0.043x - 0.053$
$R^2 = 0.0994$

Chemical	Standard	Standard	Standard	Standard	Standard	B	Leaf + water extract	Leaf + water Extract
	1	2	3	4	5	6	7	8
Tannic acid	1ml	2ml	3ml	4ml	5ml	----	1ml	1ml
Folin	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
H2O	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
Na2Co3	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml

100ml make up distilled wat

➢ *Chlorophyll Estimation (Leaf powder, Morus alba linn)*

S.No.	Wave length	Absorbance
1	645	0.510
2	663	1.177
3	652	0.657

Chlorophyll A Content	Chlorophyll B Content	Chlorophyll Total
1.357	0.647	1.974

IV. RESULT AND DISCUSSION

➢ *ORGANOLAPTIC STUDY*

Table- Organoleptic study

S.NO.	Parameters	Characteristics features
1	Color	Green
2	Order	Characteristics
3	Taste	Acrid
4	Nature	Coarse powder

Table-Extract Characteristics

Types of solvent	Consistency	Color	Extractive value% w/w
Petroleum ether	Oily	Dark green	2.75%
Chloroform	Oily	Light yellow	140.85%
Acetone	Pasty	Green	17.05%
Ethanol	Pasty	Dark green	36.60%
Methanol	Pasty	Dark green	63.70%

Table-Phyto-chemical screening

(A) ALKALOIDS-

S.No.	Plant constituents test/reagent used	Petroleum ether	Chloroform	Acetone	Ethanol	Methanol
1	Meyer's reagent	+	+	+	+	+
2	Dragendroff's reagent	+	+	+	+	+
3	Wagner's reagent	+	+	+	+	+
4	Hager's reagent	_	_	+	+	+

(B) TEST FOR CARBOHYDRETES-

ISSN No:-2456-2165

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Molisch's Test	_	+	+	+	+
Barfoed's Test	_	_	_	_	_
Test for pentoses	+	_	+	+	+

(C) TEST FOR AMINO ACIDS-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Millon's	+	_	+	+	+
Ninhydrine	_	_	_	_	_

(D) TEST FOR FLAVONOIDS-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Alkaline reagent test	_	_	_	+	+
Zinc hydrochloride test	_	+	+	_	_

(E) TEST FOR ANTHRAQUINONE GLYCOSIDES-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Test for hydroxyl	+	_	_	+	+
Bronteager's test	_	_		+	+

(F) TEST FOR CARDIAC GLYCOSIDES-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Baljets test	_	_	+	+	+
Raymond's test	_	_	_	+	-
Legal's	_	_	_	+	+

(G) TEST FOR SAPONIN GLYCOSIDES-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Forth formation test	+	_	_	-	+
Haemolysis test	_	_	_	_	_

(H) TEST FOR TANNINS (PHENOL COMPOUNDS)-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Feric chloride test	_	_	_	_	_

(I) TEST FOR STEROIDES & TRI TERPENOIDS-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Salkowski test	_	_	_	+	_
Sulfur powder test	_	_	_	_	_

(J) TEST FOR NAPTHOQUINONES-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Dam Karrer test	_	_	+	_	_

(K) TEST FOR STARCH-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Starch (Amylum)	_	_	_	+	_

Table-TLC Studies (Calculation of R_f Value)-

Extract	Non Polar						Polar					
	Mobile Phase A		Mobile Phase B		Mobile Phase C		Mobile Phase D		Mobile Phase E		Mobile Phase F	
Pt. ether	Spot no.	Rf value	Spot no.	Rf value	Spot no.	Rf value	Spot no.	Rf value	Spot no.	Rf value	Spot no.	Rf value
	2	0.62 0.44	1	0.47	1	0.62	2	0.36 0.22	2	0.83 0.51	1	0.61
Chloroform	1	0.52	1	0.33	1	0.21	1	0.52	2	0.48 0.33	1	0.84
Acetone	1	0.91	2	0.92 0.59	1	0.22	1	0.91	1	0.52	1	0.91
Ethanol	2	0.72 0.55	2	0.88 0.61	3	0.96 0.61 0.43	1	0.94	1	0.95	1	0.82
Methanol	1	0.64	1	0.32	1	0.35	1	0.44	1	0.42	1	0.59

(L) TEST FOR PROTEINS-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
Hydrolysis test	_	_	_	_	_

(M) TEST FOR FATS & FIXED OILS-

Extract	Pt. ether	Chloroform	Acetone	Ethanol	Methanol
	_	_	_	_	_

+ mean present, -mean absent

Table-Physico - chemical Parameter

S. No	Physico - chemical Parameter	Value
1	Moister value	12.5%
2	Ash value	7.5%

V. ANTIOXIDANT CONTENT

Table-Total Phenolics, Flavonoids & Tannins content of Ethanolic extracts-

Plant extracts	Total phenolics (mg gallic acid equivalent/g)*	Total flavonoid (mg rutin equivalent/g)*	Total tannin (mg tannic acid equivalent/g)*
Ethanolic extract	**3.44**	**5.00**	**3.00**

- *Chlorophyll Estimation (Leaf powder, Morus alba linn)*

S.No.	Wave length	Absorbance
1	645	0.510
2	663	1.177
3	652	0.657

Chlorophyll A Content	Chlorophyll B Content	Chlorophyll Total
1.357	0.647	1.974

ISSN No:-2456-2165

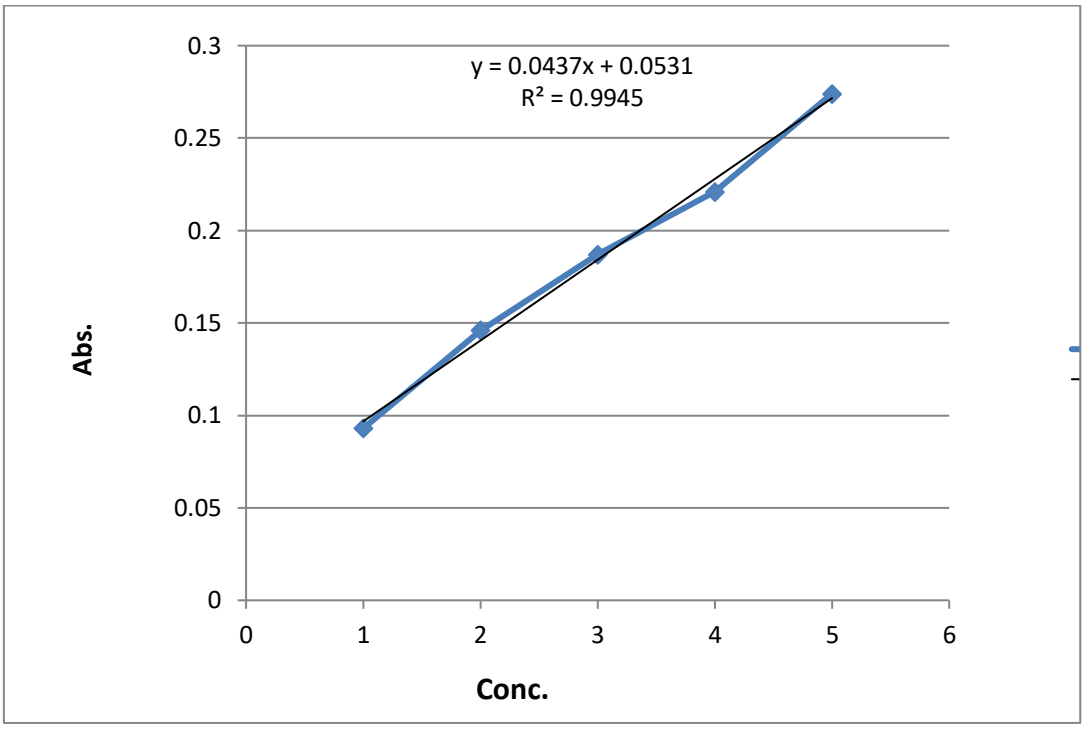

Fig 11: Standard curve of Tannin content.

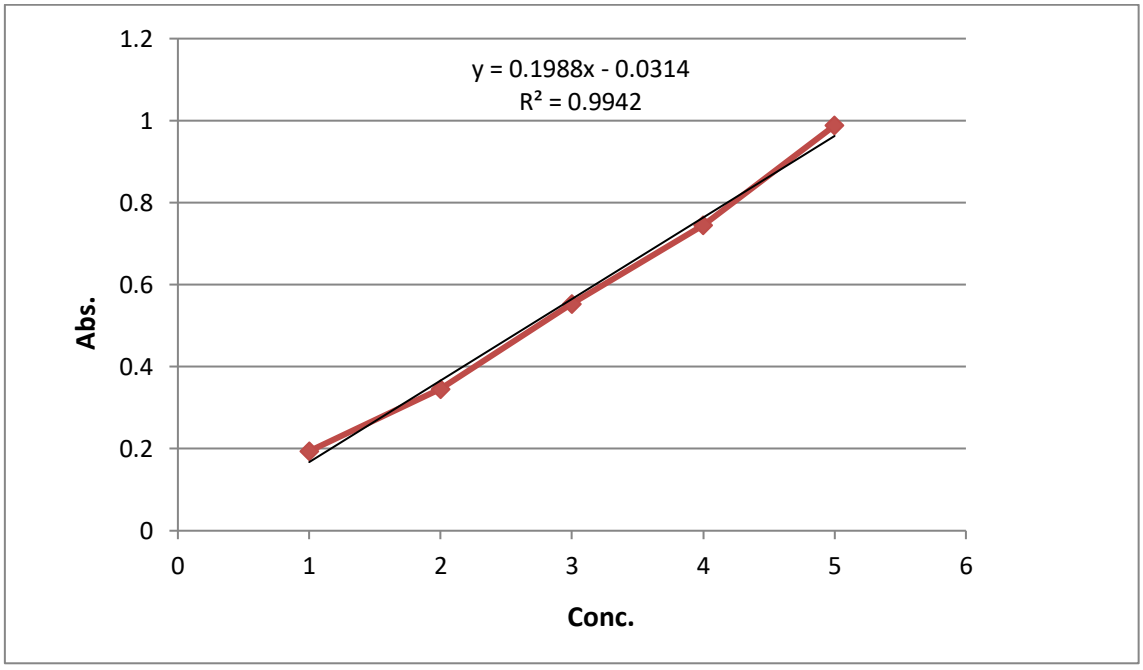

Fig 12: Standard curve of Phenol content

ISSN No:-2456-2165

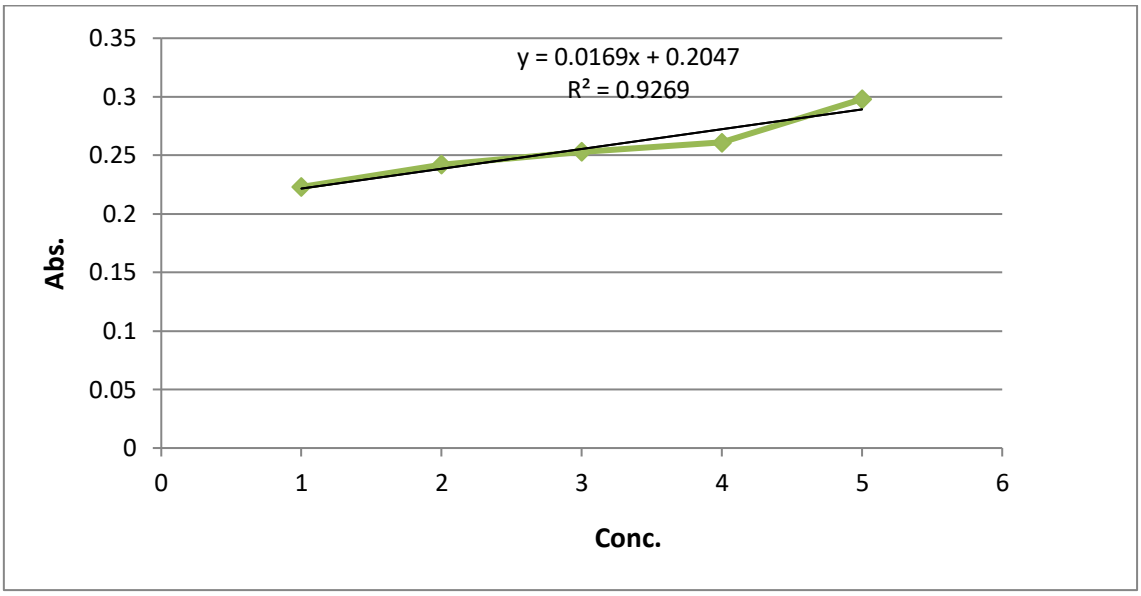

Fig 13: Standard curve of Flavonoid content

REFERENCES

[1]. Thaipitakwong, T.;Numhom, S.; Aramwit, P. Mulberry leaves and their potential effects against cardiometabolic risks: a review of chemical compositions, biological properties and clinical efficacy. Pharmaceutical Biology; 2018; 56(1); 109-118.

[2]. Sharma, A.; Krishna, V.; Kaur, P.; Rayal, R. Charaterization and screening of various Mulberry varities through morpho-biochemical characteristics. Journal of Global Biosciences; 2015; 4(1); 1186-1192.

[3]. Killedar, S. G.; Pawar, A. V. Preparation of herbal tea from mulberry leaves. Journal of Medicinal Plants Studies; 2017; 5(2): 325-328.

[4]. Kim, S. B.; Chang, B. Y.; Hwang, B. Y.; Kim, S. Y.; Lee, M. K. Pyrrole alkaloids from the fruits of Morus alba. Bioorganic & Medicinal Chemistry Letters; 2014; 24(24), 5656–5659.

[5]. Asano, N.; Yamashita, T.; Yasuda, K.; Ikeda, K.; Kizu, H.; Kameda, Y.;Ryu, K. S. Polyhydroxylated Alkaloids Isolated from Mulberry Trees (MorusalbaL.) and Silkworms (BombyxmoriL.). Journal of Agricultural and Food Chemistry, 2001; 49(9), 4208–4213.

[6]. Wang, X.; Kang, J.; Wang, H-Q.; Liu, C.; Li, B-M.; Chen, R-Y. Three new alkaloids from the fruits of Morus alba, Journal of Asian Natural Products Research, 2014; 16(5), 453-458.

[7]. Chu, Q.; Lin, M.; Tian, X.; Ye, J. Study on capillary electrophoresis-amperometric detection profiles of different parts of Morus alba L. J. Chromatogr. A 2006, 1116, 286–290.

[8]. De Freitas, M. M.; Fontes, P. R.; Souza, P. M.; William Fagg, C.;Neves Silva Guerra, E.; de Medeiros Nóbrega, Y. K.; Silveira, D.; Fonseca-Bazzo, Y.; Alberto Simeoni, L.; Homem-de-Mello, M.;Oliveira Magalhães, P. Extracts of Morusnigra L. leaves standardized in chlorogenic acid, rutin and isoquercitrin: tyrosinase inhibition and cytotoxicity. Plos One, 2016; 11(9), e0163130.

[9]. Kokate, C.K.; Practical Pharmacognosy. Delhi: VallabhPrakashan; 2008.p. 108-9.

[10]. AOAC. Official Methods of Analysis .15th ed. Washington, VA:Association of Official Analytical Chemists; 1990.

[11]. Doshi, G. M.; Zine, S. P.;Chaskar, P. K.; Une, H. D. Solicitation of HPLC and HPTLC Techniques for Determination of Rutin from *PolyalthialongifoliaThwaites*,harmacognosy Res. 2014; 6(3): 234–239.

[12]. Hussain F; Rana Z; Shafique H;Malik A;Hussain Z; "Phytopharmacological potential of different species of Morus alba and their bioactive phytochemical :A review'',Asian Pacific Journal of Tropical Biomedicine, Volume 7,Issue 10 ,October 2017,page no-950-956.

[13]. Chang,L.Y.,Li,K.T.,Yang,W.J.,Chang,J.Ch.&Chang, M.W,"Phenotypic classification of mulberry (Morus) species in Taiwanusing numerical taxonomic analysis through the characterization of vegetative traits and chilling requirements,"Scientia Horticulturae, 176. 208-217. Sep.2014.

[14]. Kamal jeet "Morus Alba Linn: A Phytopharmacological Review. Int. J. Pharmacy and Pharmaceutical Sciences" 2013; 5(2): 1-5.

[15]. Ishrat Younus , Anab Fatima , Syed Muzaffar Ali, "A Review of Ethobotany, Photochemistry, Antiviral and Cytotoxic Anticancer Potential of Morus Alba Linn". IJARR, 1(2), 2016; 84 96.

[16]. Srivastava, S., Kapoor, R., Thathola, A. & Srivastava, R.P,"Nutritional quality of leaves of some genotypes of mulberry (Morus alba)," International Journal of Food Sciences and Nutrition", 57. 305-303. Aug-Sep.2006.

[17]. Cordell, S., Goldstein, G., Mueller, D., Webb, D. & Vitousek, P.M,"Physiological and morphological variation in Metrosideros polymorpha, a dominant Hawaiian tree species, along an altitudinal gradient: the

role of phenotypic plasticity," Oecologia,113. 188-196. Jan.1998.
[18]. Pethakmsetty Lakshmi;Seru Ganapaty & K.Mary Bharathi "Chemical & biological examination of leaves of morus indica" Internatinal research journal of pharmacy Department of Botany, Andhra university Visakhapatanam, A.P. India ;approved for publication 13/05/13.
[19]. Hesham AEB, Abdel NBS, Jari S, Kalevi P. Oral supplementation of morus alba L. (Mulberry) root bark fractions, can relieve the oxidative stress and hyperlipidemia induced in hypercholesterolemic rats.
[20]. Sánchez-Salcedo, E.M., Mena, P., García-Viguera, C., Hernández,F. & Martínez, J.J, "(Poly)phenolic compounds and antioxidantactivity of white (Morus alba) and black (Morus nigra) mulberry leaves: Their potential for new products rich in phytochemicals,"Journal of Functional Foods, 18. (2015b) 1039-1046. Oct. 2015b.
[21]. Ercisli, S. & Orhan, E, "Chemical composition of white (Morus alba), red (Morus rubra) and black (M. nigra) mulberry fruits,"Food Chemistry, 103. 1380-1384. 2007.
[22]. Stone, Katharine R. 2009. Morus alba. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: http://www.fs.fed.us/database/feis/ [2009, November 30]
[23]. Gryn-Rynko A; Bazylak G;Olszewska-Slonina d; "New Potential Phytotherapeutic Obtained from White mulberry (Morus alba linn) leave.''Biomedicine & Pharmacotherapy, Volume-84,December 2016 Pages-628-636.
[24]. **Feger Josh, "White Mulberry,** (*Morus Alba*) **Interesting Information about Plant" Bellarmine University,** Page prepared by November 2005.