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PHYTOCHEMICAL AND PHYSICOCHEMICAL ANALYSIS OF *TAMARIND INDICA* LEAVES (*Tamarindus indica*)

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Abstract

According to world health organization medical plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine , which has compounds derived from medicinal plants. One of the such plant is tamarind indica which is rich in minerals too like K, P, Ca, Mg, and it contain high amount of protein and carbohydrate was taken for the present study phytochemical and physicochemical consitituents which are present in the leaves are discussed in the paper .

Keywords:- Tamarind ,Phytochemical , Physicochemical , Tannins, Proteins, Methanol, Ethanol.

INTRODUCTION

India has a great diversity of medicinal plant. There are thousands of plant which are used in traditional medicinal system to cure many diseases since thousands of year. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal system such as ayurveda. Unani and siddha A large portion of the world population especially in developing countries depends on the traditional systems of medicine for a variety of disease.

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The chemicals i.e. phytochemicals plays a vital role against number of diseases such as asthma , arthritis , cancer, etc. Unlike pharmaceuticals chemicals these phytochemicals do not have any side effects. In the last few years the search

continues for safe and effective antimicrobial agents with which a wide variety of bacterial infections can be treated. Nowadays the public is relying on alternative medicine because of its less or no side effects. So with this view in mind for the present study. I have chosen to work on

Tamarindus indica. *Tamarindus indica* fruits shows an achromatic colour that turns black or black- brown aromatic and bitter on ripening. This fruit is very much rich in certain minerals, including, potassium, phosphorus, calcium, and magnesium it contains highest amount of protein and carbohydrate then any other fruit , though it contains smaller amounts of iron and vitamin A also .

Brief Description of Tree

Tamarindus indica L is one of the chief leguminous tree species of Fabaceae family. Almost each and every part of this plant has some use in medicinal , nutritional, economic , or environment at field. Al though it is capable of tolerating a dry condition of 5-6 months long duration , but it has a very minimal amount of chance in surviving at stumpy temperatures.

Vernacular Names :-

Hindi - Imli, katare, Amli.

Tamil - Puli, Ambilam, Amilam.

Oriya- Teetuli, Tentuli.

Bengali - Tentul, Tinturiamli. Ambli, Nuli

Assames- Teteli

Classification

Kingdom – Planatae

Sub kingdom – Tracheobionta

Class- Magnoliopsida

Order- Fabales

Family- Fabaceae

Genus- Tamarindus

Species- Indica

Scientific name – *Tamarindus indica*

Morphology

Tamarindus indica L. (Family—Leguminosae) commonly known as

Tamarind grows naturally in the tropical and sub- tropical regions of India it was found trought out the tropical belt, from Africa to south Asia , Northern Australia , through out south east Asia , Taiwan and china.

The *Tamarind* flowers blooms(although inconspicuously) with red and yellow elongated flowers . Flowers are 2.5 cm (1 inch) wide , five- petalled, borne in small racemes and yellow with orange or red streaks. Buds are pink and are lost when the flower blooms.

Uses

Tamarind indica leaves reported to possess various pharmacological activities.

1- It is a natural bio preservative.

2- It is used for treat inflammation.

3- It is used for treating diabetes.

4- It is used for treating constipation.

5- It is used for treating indigestion.

6- It is used for treating flatulency.

7- It is used for treating bacterial infections.

8- It is used to treat ulcers.

9- It is used as antivenom.

10- It is treats liver disorders.

11- It is used to treat fungal infections

MATERIALS AND METHODS

Collection of Sample

Leaves of the tree were collected from Majhgawan tree leaves were cleaned, washed and dryied under shade and grounded to a fine powder by grinding it in a mixer and after sieving it was kept in the air tight container for physicochemical and phytochemical anaylsis .

The apparatus chemical, and glass ware's which were used in the study are given below.

List of Apparatus

1. Weighing machine
2. Shaker
3. Water bath
4. Hot air oven
5. Dessicator
6. Muffle furnace
7. Spatula
8. Tray dryer
9. Filter paper
10. Sieve
11. HPTLC
12. Mixer
13. Grinder machine

List of Chemicals

1- Nitric acid

2 -Chloroform

3-Sulphuric acid

4-Ferric chloride

5- Acetone

6-Sodium bi carbonate

7-Benzene

8-Distilled water

9-Ethanol

10-Methanol

11-Hydrochloric acid

List of Reagents

1. Dragendroff's reagent
2. Wagner's reagent
3. Hager's reagent
4. Anthrone reagent
5. Sodium picric reagent 6.Fehling's solution A&B

List of Glass Wares

1-Beaker

2-Pipette

3-conical flask

4-Centrifuge Tube

5-Funnel

6-Glass Rod

7-Petri Dish

8-Test tube

9.Measuring cylinder

10.Dropper

11-Crucible

12-Reagent bottle

13-Capillary tube

14-China Dish

Preparation of reagent

1-Dragendroff's reagent

Solution A- 2.5 ml. of glacial acetic acid was added in 10 ml. of distilled water and mixed thoroughly. Then Dissolved 0.2 g of bismuth subnitrate in acetic acid.

Solution B- Dissolved 4 g of potassium iodide in 10 ml. of distilled water.

Then Mixed 1 ml. of solution A, and 1 ml. of solution B, andadded 2 ml. of glacial acetic acid and 10 ml. of distilled water.

2-Wagner's reagent

Dissolved 1 g of potassium iodide in 2.5 ml. of distilled water. Added 0.65 g of iodide .Mixed until the iodine is dissolved . Added 47 ml.of distilled water.

3-Hager's reagent

This reagent was prepared by dissolving 2 gm of iodine and 6 gm of potassium iodide in 100 ml. of distilled water.

4-Fehling's solution A & B

Fehling solution A, was prepared by dissolving copper sulphate in distilled water and a few drops of sulphuric acid was added to the mixture.

Fehling solution B, is a colourless aqueous solution of Rochelle salt(the common name of potassium sodium tartrate) in an alkaline base like sodium hydroxide , it was prepared by mixing solution potassium, tartrate and sodium hydroxide in distilled water.

5-Mayer's reagent

Was freshly prepared by dissolving a mixture of mercuric acid chloride (1.36 g) and 5.00 g of potassium iodide in water.

6-Anthrone reagent

Dissolved 2 g of Anthrone in 1 litre of concentrated sulphuric acid.

Physicochemical analysis

The parameters which were analysed in the present study are.

A- Ash value.

B- Extractive value.

A-Ash value :-

Ash values are useful in determining the quality and purity of crude drugs , especially in powder form the objective of ashing is to remove all the traces or organic matter , which may otherwise interfere in an analytical determination , on incineration crude drugs normally leave an ash usually consisting of carbonates, phosphates, and silicate of sodium, potassium, calcium and magnesium.

(i)Total ashvalue:-

2 or 3 g of sample, weighed of the ground sample in a silica dish previously ignited and weighed, scattered the sample powder in a fine even layer on the bottom of the dish , Incinerated gradually by increasing the heat not exceeding dull red heat until free from carbon, coal, and weighed.

Average = final weight – Initial weight of crucible/ Total no. of sample

Total ash = Average × 100 / weight of sample taken

(ii)Acid Insoluble ash value:-

To the crucible containing total ash, added 25 ml. of dilute HCL. The insoluble matter was collected on the ash less filter paper (whatman no. 42) and washed with hot water. The filter paper containing insoluble matter was transferred to the previously weighed crucible and dried it on a hot plate and ignited .Residue was allowed to cool in a desiccator for 30 minutes and was weighed.

Preparation of the extract

5 g of sample was macerated with 100 ml. of chloroform and it water in a closed flask for 24 hours, shaking frequently during 6 hours and allowed it to stand for 18 hours.

B-Extractive value:-

This method is used to determine the amount of active consitituents in the given sample.

1-N-butene alcohol soluble extractive value:-

10 ml. of N-butene alcohol soluble extract of sample was taken in a previously weighed petri dish and kept it in a water bath for six hours and then cooled at room temperature and then in desiccator and than weighed the petri dish.

N-butene alcohol soluble extractive value = Weighed petri dish with sample – Weighed petri dish without sample.

2- Water soluble extractive value :-

10 ml. of Water soluble extract of sample was taken in a previously weighed petri dish and kept it in a water bath for six hours and then cooled at room temperature and then in desiccator and than weighed the petri dish.

Water soluble extractive value = Weighed petri dish with sample – Weighed petri dish without sample.

3-Acetone soluble extractive value :-

10 ml. of Acetone soluble extract of sample was taken in a previously weighed petri dish and kept it in a water bath for six hours and then cooled at room temperature and then in desiccator and than weighed the petri dish.

Acetone soluble extractive value = Weighed petri dish with sample – Weighed petri dish without sample.

4- Methanol soluble extractive value:-

10 ml. of Methanol soluble extract of sample was taken in a previously weighed petri dish and kept it in a water bath for six hours and then cooled at room temperature and then in desiccator and than weighed the petri dish.

Methanol soluble extractive value = Weighed petri dish with sample – Weighed petri dish without sample.

5- Ethanol soluble extractive value:-

10 ml. of Ethanol soluble extract of sample was taken in a previously weighed petri dish and kept it in a water bath for six hours and then cooled at room temperature and then in desiccator and than weighed the petri dish.

Ethanol soluble extractive value = Weighed petri dish with sample – Weighed petri dish without sample.

6-Petroleum ether soluble extractive value:-

10 ml. of Petroleum ether soluble extract of sample was taken in a previously weighed petri dish and kept it in a water bath for six hours and then cooled at room temperature and then in desiccator and than weighed the petri dish.

Petroleum ether soluble extractive value = Weighed petri dish with sample – Weighed petri dish without sample.

7-Benzene soluble extractive value:-

10 ml. o f Benzene soluble extractive of sample was taken in a previously weighed petri dish and kept it in a water bath for six hours and then cooled at room temperature and then in desiccator and than weighed the petri dish.

Benzene soluble extractive value = Weighed petri dish with sample – Weighed petri dish without sample.

Phytochemical analysis

In this the following parameters were analysed according to various procedures given in literature.

1- Carbohydrates

2- Flavonoids

3- Proteins

4- Alkaloids

5- Glycosides

6- Phenols

7- Steroids

8- Resins

9- Saponin

Preparation of Extract

2 gram of powdered sample ***Tamarind indica***

(*Tamarind indica*)was mixed will with 100 ml of distilled water and alcohol separately and it was kept in rotatory shaker for 48 hours in incubation finally filtered it by using whatman no. 42 filter paper and the above filtrate was stored in refrigerator and used for the analysis.

1-Test for Carbohydrate

(a) Anthrone test

0.5 ml. of aqueous extracts of sample was shaken with 2.5 ml. of water, and filtered and then filtrate was concentrated. To this 1.25 ml. of anthrone reagent solution was added and noted the change.

(b) Fehling's test

Taken 2 ml. of the sample solution in a clean test tube. Added 2 ml of Fehling's solution A and Fehling's solution B to it. Kept the solution in a boiling water bath for about 10 minutes. and noted the change.

2-Test for Flavonoids

(a)Shinoda's test
To 0.5 ml. of ethanol extract of sample a piece of metallic magnesium was added. Followed by addition of 2 drops of concentrated hydrochloric acid and observed.

(b)Alkaline test

To 0.5 ml. of ethanol extract of sample, added few drops of sodium hydroxide solution and noticed the change.

3-Test for Protein

(a)Biuret test
To 0.5 ml. of aqueous extract of sample, added10% NaOH solution and few drops of 1% CuSO4 and observed the change.

(b)Ninhydrin test

To 0.5 ml. of aqueous extract of sample, added few drops of 5 % ninhydrin and then boiled and noticed the change.

(C)Xantho protein test

Taken 1 ml. of aqueous extract of sample and added 2 ml. of distilled water and 0.5 ml., of concentrated nitric acid and observed.

4-Test for Alkaloids

(a)Dragendroff's test

To 0.5 ml. of ethanol extract of sample Dragendroff's reagent was added.(potassium bismuth iodide solution)and noted the change. (b)Mayer's test

To 0.5 ml. of ethanol extract of sample Mayer's reagent was added.(potassium mercuric iodide solution) and noted the change.

(c)Wagner's test

To 0.5 ml. of ethanol extract of sample the Wagner's reagent was added.(solution to iodide in potassium iodide) and observation were made.

(d)Hager's test

To 0.5 ml. of ethanol extract of sample, few drops of Hager's reagent was added and observed the change .

5-Test for Glycosides

(a)Baljet test

Added 2 drops of sodium picric reagent in 2 ml. of ethanolic extract of the sample and observed the change.

(b)Keller killani test

To the ethanol sample extract added 0.4 ml. of glacial acetic acid containing traces of ferric chloride and 0.5 ml of concentrated sulphuric acid and observed the change

6-Test for Phenols

To 1 ml. of aqueous extract of sample, added 2 ml. of distilled water followed by few drops of Fecl3 observed the change.

7-Test for Steroids

(a)Salkowski's test

Added 1 ml. of concentrate sulphuric acid to 2 ml. of chloroform extract of sample from the side of test tube and noted the change.

8Test for Resins

0.5 ml. of aqueous extract of sample was treated with a few drops of acetic anhydride solution followed by 1 ml. of concentrated sulphuric acid. and change was noted.

9-Test for Saponins

(a)Foam test

1 ml. of aqueous extract of , was shaken with distilled water and observation were made.

Quantitative analysis Preparation of extract

To 3 gm of sample added 30 ml. methanol and shaked for 4-6 hours, then heated on water bath till it thickens, then spot was applied on TLC plate and then placed in solvent (mixture of ethyl acetate and toluene in ratio 7:3) the dipped spot runs on the TLC plate and after the run TLC plate was taken out and it was left to dry and then the dried plate was washed with sulphuric acid and kept for drying and obseverbed the dip run with spectrophotometer at 244/366 nm wavelength.

Method of High Performance Thin layer Chromatography(HPTLC)

Sample on a TLC plate was run by dipping the plate in a particular system. The compounds in the sample will then separate based on their polarities. HPTLC uses a computer system connected to an autosampler a scanner. The auto-sampler spots the specified amount of sample on the plates ,after which the plates can be developed in a glass chamber or an automatic developing chamber. Once the chromatogram run is complete, the plates are dipped in the derivatization agent either manually or using a chromatogram immersion device. The derivatization agent can be sprayed on the plate using a derivatizer. The compounds are quantified by scanning the plate using a scanner.

Derivatization

1- Colorless or non-UV absorbing substances can be detected by derivatization.

2- Derivatization with liquid reagents requires spraying or dipping of the plate.

3- The possibility of derivatization the separated zones stored on the plate is a special advantage of thin layer chromatography.

4- Immersion device.

5- TLC Sprayer.

6- TLC Plate Heater.

RESULTS AND DISCUSSION

In the present study the sample were screened for Total ash value, Acid soluble extractive value, n-butene alcohol soluble extractive value, Water soluble extractive value, Acetone soluble extractive value, Methanol soluble extractive value, Ethanol soluble extractive value, Petroleum ether soluble extractive value, Benzene soluble extractive value and the results are given in tables 1 to 9 below respectively..

1-Physicochemical Analysis

Ash value

Table no. 1:- Total ash value in 2 gm of sample.

S.NO.	Weight of empty crucible(A)	Sample	1st day weight (B)	2nd day weight (C)	3rd day weight (D)	Difference (D – A)
1.	18.3870	2 gm	18.7311	18.7294	18.7271	0.3401
2.	15.0799	2 gm	15.4145	15.4132	15.4084	0.3285
	Average	-	-	-	-	0.3343

Total Ash value = Average ×100/ Sample weight

Total Ash value= 0.3343×100/2

= 16.71 %

Table no. 2:- Acid soluble Ash value in 2 gm sample.

S.NO.	Weight of empty crucible(A)	Sample	1st day weight (B)	2nd day weight (C)	3rd day weight (D)	Difference (D – A)
1.	18.3870	2 gm	18.4193	18.4187	18.4186	0.0316
2.	15.0799	2 gm	15.1148	15.1141	15.1141	0.0342
	Average	-	-	-	-	0.0329

Total insoluble Ash value = Average× 100/Sample weight

Total insoluble value = 0.0329 ×100/2

= 1.645 %

Extractive Value

Table no. 3:- n- butene alcohol soluble extractive value of *Tamarind indica*.

S.NO.	Petri dish pre weight(A)	Sample	Petri dish final weight (B)	Difference (B – A)
1.	31.6190	10 ml.	31.6689	0.0499
2.	30.6044	10 ml.	30.6612	0.0568
	Average	-	-	0.0553

Extractive value = Average × 500

Extractive value = 0.0533× 500

= 26.65 %

Table no. 4:- Water soluble extractive value of *Tamarind indica*.

S.NO.	Petri dish pre weight(A)	Sample	Petri dish final weight (B)	Difference (B – A)
1.	36.1263	10 ml.	36.2900	0.1637
2.	34.6843	10 ml.	34.7041	0.0201
	Average	-	-	0.0919

Extractive value = Average × 500

Extractive value = 0.0919× 500

= 45.95 %

Table no. 5:- Acetone soluble extractive value of *Tamarind indica*.

S.NO.	Petri dish pre weight(A)	Sample	Petri dish final weight (B)	Difference (B – A)
1.	33.2427	10 ml.	33.2933	0.0506
2.	32.8523	10 ml.	32.9195	0.0672
	Average	-	-	0.0589

Extractive value = Average × 500

Extractive value = 0.0589 × 500

= 29.45 %

Table no. 6:- Methanol soluble extractive value of *Tamarind indica*.

S.NO.	Petri dish pre weight(A)	Sample	Petri dish final weight (B)	Difference (B – A)
1.	33.1392	10 ml.	33.2059	0.0667
2.	32.3042	10 ml.	32.3603	0.0561
	Average	-	-	0.0614

Extractive value = Average × 500

Extractive value = 0.0614 × 500

= 30.7 %

Table no. 7:- Ethanol soluble extractive value of *Tamarind indica*.

S.NO.	Petri dish pre weight(A)	Sample	Petri dish final weight (B)	Difference (B – A)
1.	36.1271	10 ml.	36.1896	0.0625

2.	35.3704	10 ml.	35.4344	0.064
	Average	-	-	0.06325

Extractive value = Average × 500

Extractive value = 0.06325 × 500

= 31.62%

Table no. 8:- Petroleum ether soluble extractive value of *Tamarind indica*.

S.NO.	Petri dish pre weight(A)	Sample	Petri dish final weight (B)	Difference (B – A)
1.	32.8532	10 ml.	32.8742	0.021
2.	31.6190	10 ml.	31.6685	0.0495
	Average	-	-	0.03525

Extractive value = Average × 500

Extractive value = 0.03525 × 500

= 17.62%

Table no. 9:- Benzene soluble extractive value of *Tamarind indica.*

S.NO.	Petri dish pre weight(A)	Sample	Petri dish final weight (B)	Difference (B – A)
1.	27.6532	10 ml.	27.6723	0.0191
2.	25.1625	10 ml.	25.1884	0.0.259
	Average	-	-	0.0225

Extractive value = Average × 500

Extractive value = 0.0225 × 500

= 11.25 %

The results tabulated in table, 1 to 9 for ash value , Acid insoluble ash value, n-butene soluble extractive value, Water soluble extractive value, Acetone soluble extractive value , Methanol soluble extractive value , Ethanol soluble extractive value , Petroleum ether soluble extractive value , Benzene soluble extractive value, is 16.71%, 1.64%, 26.65%,45.95%,29.45%,30.7%,31.62%,17.62%,11.25% respectively.

Phytochemical Analysis

The phytochemical analysis of the sample in given below in table no.

10. phytochemical analysis of *Tamarind indica*

S.NO.	Test	Observation	Ethanol	Aqueous
1.	**Carbohydrates** (A)Anthrone Test (B)Fehling's Test	 No change No change	 N.D. N.D.	 - -
2.	**Flavonoids** (A)Shinoda's Test (B)Alkaline Test	 No change No change	 - -	 N.D. N.D.
3.	**Protein** (A)Biuret Test (B)Ninhydrin Test (C)Xantho protein Test	 No change No change Violet red colour	 N.D. N.D. N.D.	 - - +
4.	**Alkaloids** (A)Dragendroff's Test (B)Mayer's Test (C)Wagner's Test (D)Hager's Test	 No change No change Brawn colour No change	 - - + -	 N.D. N.D N.D. N.D.

5.	**Glycosides** (A)Baljet Test (B)Keller killani Test	Orange colour No change	+ -	N.D. N.D.
6.	**Phenols**	No change	N.D.	-
7.	**Steroids**	Red color	+	N.D.
8.	**Resins**	No change	N.D.	-
9.	**Saponin** (A)Foam Test	Appearance of foam	N.D.	+

'N.D'. - Not done.

'+' - Positive

'- ' - Negative

The table no. 10 reveals the following results .

1. Carbohydrate two tests were performed on the aqueous sample extract i.e.anthrone and fehling's test for testing the presence of carbohydrate both the tests gave negative results showing absence of carbohydrate.

2. Flavonoids ethanolic extract of sample was subjected to shinoda's test and alkaline test for testing the presence of flavonoids both the test gave -ve results showing absence of flavonoids.

3. For determination of protein three tests were performed on the aqueous extract of sample i.e. biuret test, xantho test and ninhydrin test positive response was given in xantho test which confirms the presence of protein and other two tests gave – ve results showing absence of proteins.

4. Ethanolic extract of the sample was subjected to dragendroff tests, wagner's tests, hager's tests and mayer's tests and the sample with wagner';;s tests gave positive response confirming the presence of alkaloids while all other three tests gave negative results showing the absence of alkaloids tests.

5. Ethanolic extract of the sample was subjected to baljet test and kellerkillani tests positive response was given in baljet test which confirms the presence of glycosides while the other test gave negative results showing absence of glycosides

6. Aqueous extract of the sample gave greenish blue colour with ferric chloride solution which confirms the presence of phenols.

7. The ethanolic extract of the sample was subjected to salkowsains tests and it gave positive results confirming the presence of steroids.

8. Aqueous extract of the sample gave negative response with acetic acid, anhydride solution followed by 1 ml. of concentrate sulphuric acid confirming the absence of resins.

9. The aqueous extract of the sample the test gave positive response for foam test which confirms the presence of saponin test.

Quantitative Analysis"HPTLC Fingerprint profile
Tamarindus indica "

Table no.11:- Rf value of HPTLC Fingerprint profile on *Tamarind indica*:

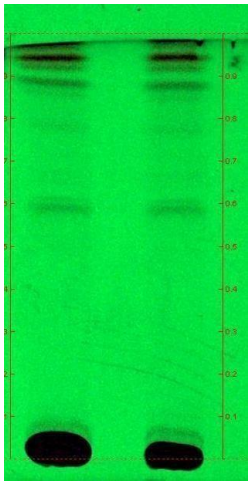
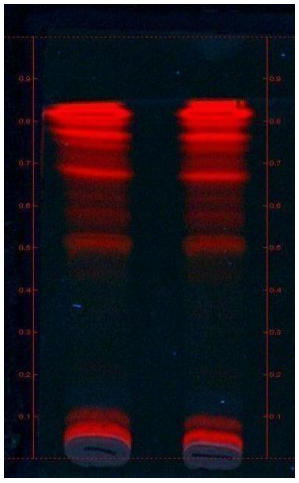
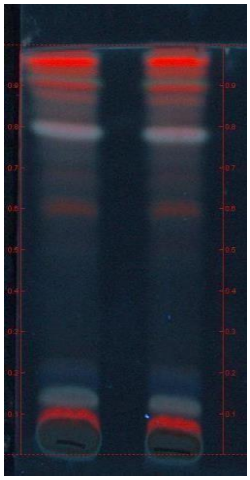
S.NO.	Rf Values	254nm before derivatization	366nm before derivatization	366nm after derivatization
1.	Rf 1	0.1(green)	0.1(red)	0.1(navy blue)
2.	Rf 2	0.3(sky blue)	0.6(navy blue)	0.2(sky blue)
3.			0.7(violet)	0.4(violet)

Table no. 11. shows different spots at 254 nm before derivatization and 366 nm before and after derivatization.

(a)At 254nm(before derivatization):- Fine spots were observed at 0.1(green), 0.3(sky blue.

(b)At 366nm (before derivatization):- 3 spots were observed they are 0.1(red), 0.6(navy blue), 0.7(violet.

(c)At 366nm (after derivatization) :- 3 spots were observed are 0.1(navy blue), 0.2(sky blue), 0.4(violet).

254nm **366(before)** **366(after)**

CONCLUSION

According to world health organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants Should be investigated for better understanding for their properties. safety and efficiency. So seeing the results and availabity of *Tamarind*, the extract of *Tamarind* leaf powder, can be made on large scale by spenting very meagre amount and one can become self employed and can support some other families too.

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