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Pharmacognostic, phytochemical and antioxidant activity of dried bark of *Tamarindus indica* L

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Abstract
Tamarindus indica L is a tropical plant that is used around the world as traditional medicine. Bark products constitute nearly one third of plant material used in Indian traditional medicine. Since the large majority of Indians make use of traditional health care, bark is fundamental to the traditional pharmacopoeia. Yet no scientific studies were reported on the dried outer bark of the plant. Hence the present study was carried out to assess the pharmacognostic, phytochemical and antioxidative potential of *T. indica* L bark. The bark was harvested and then dried in the absence of direct sunlight. The extraction was done by cold perculation method and the methanol extract was stored in dry bottles. Pharmacognacy evaluation includes organoleptic, anatomical and proximate analysis. Phytochemical analysis revealed the presence of Alkaloids, Phenols, Terpenoids, Saponinns, Flavonoids, Tannins, Glycosides and Steroids and the total flavonoid content was also estimated from the bark as 24.81µg/ml. FTIR analysis of the bark revealed the presence of most important bioactive metabolites. The antioxidant assay such as DPPH, phosphomolybneum nitric oxide scavenging assay revealed significant IC_{50} value 83.45% at 20µg/ml in dried bark of *Tamarindus indica*. The activity was does depended in nature.

Keywords: *Tamarindus indica* L, bark, Ant oxidative activity, menthol extract

Introduction
Tamarindus indica L. or tamarind, as it is commonly known, is a medium-sized tree belonging to the *Caesalpinaceae* family. Tamarind has been used for centuries as a medicinal plant; its fruits are the most valuable part which has often been reported as curative in several pharmacopoeias. Nevertheless, other parts especially bark have been less studied. It is well known that different climatic, ground and growing conditions can modify qualitatively and quantitatively the chemical composition of the plant and therefore its pharmacological uses [1].
Research of plants that have antioxidative activity has become a topic of increasing interest in light of the important roles for antioxidative compounds in the treatment and prevention of pathologies linked to oxidative stress that is generated by free radicals [2]. Indeed, antioxidants help to neutralize free radicals which can damage cellular membranes and interact with the genetic material of cells. Antioxidants from fruits, vegetables and beverages play an important role in human health, for example preventing cancer and cardiovascular diseases, and reduce the incidence of different diseases [3]. At the chemical level, prior research has revealed the presence of phenolic constituents such as flavonoids, alkaloids, and tannins [4].

Materials and Methods
Collection and authentication of plant material:
Dried bark of *Tamarindusindica* were identified and collected from local area Minjur Lakshmi puram at the period of November 2019. It was further authenticated by taxonamist Department of Botany Queen Mary's college Chennai.

Preparation of extract
The freshly collected stem bark was chopped into pieces and Shade dried at room temperature (27-30ºC) to constant weight for two weeks. 200g of each of the bark was coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers. 30g of bark powdered was extracted with 100ml of methanol and soaked for 3 days. After 3 days the extract was filtered through the Whatman No. 1 filter paper. The extract was transferred into air tight container until further use.

Macroscopic Study
Macroscopic characters of dried bark of *Tamarindus indica* were analyzed systematically and its morphological characters like size, shape etc., were noted down.

Microscopic Study
The microscopic study is the anatomical study which is done by taking appropriate section of the bark under study. Each distinguishing character can be noted down, some of which are retained in the powder study also. Some of the chemicals which are used in obtaining clear sections are safranine, methyl orange, etc. Thin sections of bark was prepared and stained with 0.1% of saffranin and mounted in glycerin. These sections were observed under compound microscope and microphotographs were taken with the help of camera.

Organoleptic Study
Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality

of a particular drug. Bark was evaluated for its organoleptic characters like texture, taste, odour and colour etc [5].

Physicochemical analysis

The parameters which are studied are moisture content, loss On drying and water holding capacity etc. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent [methanol] yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not [6].

Loss on drying

About 10g of plant material was taken in a tarred glass and heated at 105 ± 1^0C in an oven and weighted. This procedure was reported till a constant weight was obtained. The moisture content of the sample was calculated as percentage with reference to the shade dried material as follows

$$\text{Loss on drying \%} = \frac{\text{Loss in weight}}{\text{Weight of crude drug}} \times 100$$

Water holding capacity

About 5g of plant material was taken in a conical flask to this 50ml of distilled water was added. The solution was aside for 1hr and water holding capacity was estimated.

Phytochemical analysis

Qualitative chemical tests

Test for Alkaloids

To 200µl of bark extract, (4-5) drops of concentrated hydrochloric acid, picric acid few drops was added formation of yellow precipitate indicates the presence of alkaloids.

Test for Phenols

To 200µl of bark extract, (4-5) drops of methanol, few drops of ferric chloride was added solution was observed formation of dark green color which indicates the presence of phenols.

Test for Terpenoids

To 200µl of bark extract, 2ml of chloroform, 1ml of concentrated sulphuric acid was added formation of red ring in the interface. This indicates the presence of terpenoids

Test for Saponins

To 200µl of bark extract, few ml of distilled water and mixed well. The solution was shaken for 15minutes and observed for the presence of foam which confirms the presence of saponins.

Test for Flavonoids

To 200µl of bark extract, sodium hydroxide solution few ml was added. The solution was observed the formation of yellow color, which indicates the presence of flavonoids.

Test for Tannins

To 200µl of bark extract, few drops of methanol, few drops of lead acetate was added. Formation of white precipitate indicates the presence of tannins.

Test for glycosides

To 200µl of bark extract, few drops of concentrated sulphuric acid, sodium nitropurside solution was added. The formation of blood red color in the sample indicates the presence of glycosides.

Test for Steroids

To 200µl of bark extracts, few drops of concentrated sulphuric acid, few drops acetic anhydride was added. The formation of brown color which indicates the presence of steroids.

Quantitative analysis

Determination of total falconoid by spectrometric method

To 500µl of plant extract, equal volume of methanol was added. The whole solution was allowed to stand for 5 minutes. To this 5% sodium nitrate and 10% of aluminum chloride was added. Small pellet of sodium hydroxide was also added to the test solution. After this test solution was read (510nm) in spectrophotometer for the detection of flavonoids [7].

FTIR Analysis

Fourier Transform Infrared Spectroscopy

The FTIR spectra were recorded for the methanol extract of *Tamarindus indica*. The spectrum showed various peaks in different range. The FTIR spectrum of the extract was recorded in the IR region from 4000 to 500cm^{-1} maximum of (27) scans where accumulated for each spectrum using the Horizontal Attenuated Total Reflection (HATR) device, using a shimadu FTIR 100 spectrometer. The total flavonoids were determined by FTIR method using the intensity of peak at 1741cm^{-1}. Flavonoid content was estimated and it was compared by standard Quercitin equivalence [8].

Anti-Oxidant Activity

DPPH radical scavenging activity

The antioxidant activity of sample was measured on the basis of the stable 1, 1- diphenyl 2- picrythydrazyl (DPPH) free radical scavenging activity. One ml of 0.1 Mm DPPH solutions in methanol was mixed with 1ml of various concentrations (20-120µl/ml) of essential oil. The mixture was then allowed to stand for 30 min incubation in dark. One ml methanol mixed with 1ml DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis at spectrophotometer at 517nm. Ascorbic acid was used as standard reference. The percentage of inhibition and the IC_{50} value was calculated using the following formula [9].

$$\%\text{of DPPH radical inhibition} = \left[\frac{\text{Control} - \text{sample}}{\text{Control}}\right] \times 100$$

Phosphomolybdenum Assay

The anti-oxidant activity was evaluated by reduction assay method which was based on the formation of green phosphomolybdenum complex. various concentrations of extracts were (20-120µl/ml) combined with 1ml of reagent solution (4mM ammonium molybdate, 28mM sodium phosphate and 0.6 M sulphuric acid). The tubes were capped and incubated in a water bath at 95ºC for 90 minutes. The samples were cooled to room temperature and the absorbance of the mixture was measured at 695nm against blank.

$$\% \text{ Inhibition} = \left[\frac{(\text{Abs (sample)} - \text{Abs (control)})}{\text{Abs (sample)}} \right] \times 100$$

Nitric Oxide Scavenging Assay

The methanolic extract of *Tamarindus indica* bark was taken at various concentrations (20-120µl/ml) in test tube and made up to 1ml using methanol. To this 0.03g of sodium nitropursside was dissolved in 20ml phosphate buffer at PH (7.4). All the test tubes were incubated with 29ºC for 3hours in room temperature. Griess reagent was prepared by dissolving 0.2g of sulphanilamide, 0.02g of napthylenediamine, 400µl of orthophosphoric acid. Above the solution was dissolving in 20ml of distilled water. To this 1ml of Griess reagent in each test tube added and absorbance of the solution were read at 550nm. The percentage inhibition the IC_{50} value was calculated using sodium nitropursside and Griess reagent as the control [10].

$$\% \text{ inhibition} = \left[\frac{(\text{Abs (control)} - \text{Abs (sample)})}{\text{Abs (control)}} \right] \times 100$$

Results

Organoleptic Study

Bark was evaluated for its organoleptic characters like texture (hard), taste (astingent), odour (aromatic) and colour (reddish brown).Physico-chemical Parameters like loss on drying (5.3%), water-soluble extractive (30%) and pH values (5.8) were determined as per the API guidelines. (Table: 1).

Table 1: Organoleptic characters of dried bark of *Tamarindusindica.*

S. No	Parameters	Characters
1	Texture	Hard
2	Colour	Reddishbrown
3	Taste	Astingent
4	Odour	Aromatic

Microscopic study

Anatomical studies in bark of *Tamarindus indica*

The significant anatomical details of the bark have been thoroughly studies and recorded. Transfer section of *Tamarindusindica* bark showed the presence of distinct phelloderm, uni to multiseriate rays, group of stone cells, lignified narrow fibre cells, and thick walled tanniferous cells.

Phytochemical screening

Preliminary phytochemical screening of *Tamarindus indica* showed the presence of Alkaloids, Terpenoids, Saponins, Flavonoids, Tannins, Glycosides and Steroids

Quantitative analysis of flavanoid

In *Tamarindus indica* quantitative estimation of flavonoid Was carried out in methanol bark extract as per the standard procedures. The methanol bark extract contain 24.81µg/ml of flavanoid. And data were recorded [11].

FTIR

The FTIR spectrum was recorded for methanolic bark extract ranging from 600cm^{-1} to 3800cm^{-1}. The various peaks recorded are 777.7, 888.8, 1029.2, 1444.2, 1261.2, 1314.7, 1420.2, 1458.0, 1508.1,151,9, 1608.6, 2002.6, 2089.1, 2121.3, 2850.3, 2918.2, 3253.8, and 3325.2 in different wavelength. The bands in the 3325.2 cm-1 regions were assigned to different OH functional groups (from carboxyl to flavanoids). Those between 2850.3cm^{-1} belong to streching vibrations of the aldehyde group and aromatic –CH group. The aromatic character of a compound was confirmed by the absorption band at 1608.6 cm^{-1} along with the intense absorbtion at 777.7cm^{-1}. The bands inbetween 1261.1cm^{-1} represent the C-O group deformatuion vibrations of flavanoids and carboxyl groups. The broad peak at 3253.8cm^{-1} indicates the presence of flavanoids. The sharp at 2121.3cm^{-1} confirms the presence of carbodiamide. The narrow peak at 1541.9cm^{-1} indicates the presence of nitro compounds. The narraow peak at 1458.0 the presence of methylene group. The peak at 1420.2cm^{-1} indicates the presence of carboxylic acid. The peak at, 1314.7cm^{-1} confirms the presence of sulphate groups. The narrow peak at 1144.2cm^{-1}confirms the presence of tertiary alcohols. The peak at 1029.2 cm^{-1} indicates the presence of secondary alcohols. The narrow peak at888.8cm^{-1} indicate the presence of alkenes [Spectrum-1 & Table-2].

Table 2: Qualitative Screening of Phytochemicals of Methanolic Extract of *Tamarindus indica* Linn Bark**.**

S. No	Phytochemical Constituents	Methanolic Bark Extract of Tamarindus Indica
1	Alkaloids	+
2	Phenols	-
3	Terpenoids	+
4	Saponins	+
5	Flavanoids	+
6	Tanins	+
7	Glycosides	+
8	Steroids	+

Spectrum 1: Ftir Analysis of Bark of *Tamarindus indica* Linn Bark

Table 3: FTIR Analysis

Peak list	Centre	Area	Height	Left edge	Right edge
1	777.7	-0.0	-66.2	777.7	777.7
2	888.8	-0.0	-75.2	888.8	888.8
3	1029.2	-0.0	-56.9	1029.2	1029.2
4	1144.2	-0.0	-71.6	1144.2	1144.2
5	1261.1	-0.0	-71.2	1261.1	1261.1
6	1314.7	-0.0	-69.6	1314.4	1314.7
7	1420.2	-0.0	-75.1	1420.2	1420.2
8	1458.0	-0.0	-75.1	1458.0	1458.0
9	1541.9	-0.0	75.5	1541.9	1541.9
10	1608.6	-0.0	-67.5	1608.6	1608.6
11	2089.1	-0.0	-93.5	2089.1	2089.1
12	2121,3	-0.0	-93.4	2121,3	2121,3
13	2850.3	-0.0	-82.1	2850.3	2850.3
14	2918.2	-0.0	-79.4	2918.2	2918.2
15	3253.8	-0.0	-77.1	3253.8	3253.8
16	3325.2	-0.0	-76.6	3325.2	3325.2

Anti-oxidant activity

DPPH radical scavenging activity

The anti-oxidant potential of methanol bark extract of *Tamarindus indica* was studied by DPPH assay at different concentration ranging from 20µl/ml to 120µl/ml. The anti-oxidant activity of bark extract was expressed as IC_{50}. The effective concentration of extract required to scavenge the DPPH radical by 50%. The results were compared with known antioxidant caffeic acid (IC_{50}-50mM/ml). The IC_{50} value of the standard was recorded as 85.44% at50mM/ml. The IC_{50} value of the methanol bark extract was found to be 82.47% at80µl/mg The DPPH radical-scavenging activities of the extracts depicted a dose–response relationship [Table-4 & Fig.-1].

Table 4: Antioxidant Assay (DPPH Assay)

S. No	Concentration µg/ml	OD Value	%
1	20	0.097	36.218
2	40	0.074	44.672
3	60	0.070	62.814
4	80	0.069	82.474
5	100	0.060	90.546
6	120	0.056	103.092
7	Control	0.134	-
8	IC_{50}	-	48.50

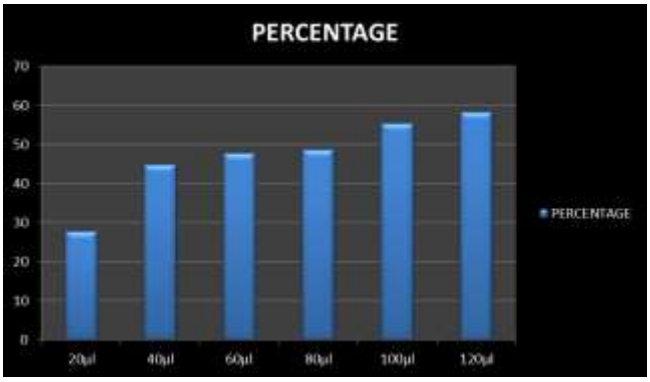

Fig 1: DPPH Assay

Phosphomolybdenum Assay

The methanol bark extract of *Tamarindus indica* was studied by the Phosphomolybdenum assay at different concentration ranging from 20µg/ml to 120µl/ml. The activity of bark extract was expressed as IC_{50} values. The effective concentration of extract required to scavenge the Phosphomolybdenum by 50%. The results were compared with known standard. The IC_{50} value of the methanol bark extract was found to be 22.43% at20µl/mg [Table-5 & Fig.-2].

Table 5: Phosphomolybdnum Assay

S. No	Concentration µG/ML	OD Value	%
1	20	0.157	22.431
2	40	0.192	37.285
3	60	0.218	49.924
4	80	0.220	66.166
5	100	0.229	80.634
6	120	0.250	92.014
7	Control	0.087	-
8	IC_{50}	-	44.58

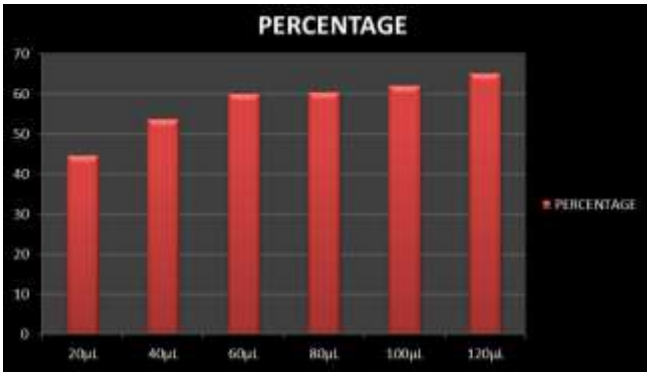

Fig 2: Phosphomolybdnum Assay

Nitric oxide scavenging assay

The antioxidant potential of methanol extract of *Tamarindus indica* was studies by nitric oxide scavenging as at different concentration ranging from 20µg/ml to 120µg/ml. The antioxidant activity of bark extract was expressed as IC_{50} values. The effective concentration of extract required to scavenging the nitric oxide reducing power by 50%. The result was compared with known standard. The IC_{50} value of the methanol bark extract was found to be 99.20% at 80µg/ml [Table-6 & Fig.-3].

Table 6: NITRIC Acid Scavenging Assay

S. No	Concentration µG/ML	OD Value	%
1	20	2.461	0.5606
2	40	2.451	0.9696
3	60	1.144	33.57
4	80	1.477	40.32
5	100	1.451	41.37
6	120	1.438	41.89
7	Control	2.475	-
8	IC_{50}	-	48.50

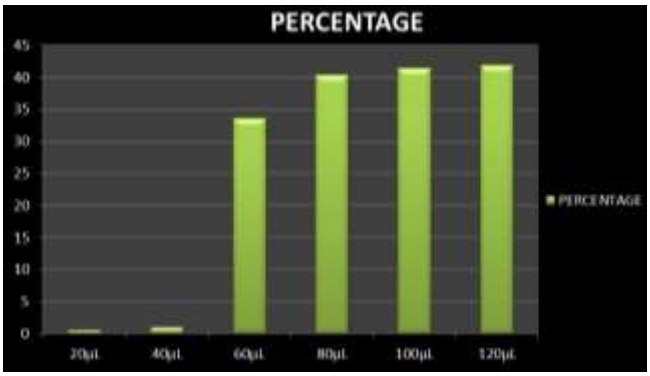

Fig 3: Nitric Oxide Assay

Discussion

Medicinal plants have enormous ability to synthesize wide variety of secondary metabolites with antimicrobial potential. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [12]. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [13]. The present study revealed that the methanolic bark extract of Tamarindus *indica* contained some secondary metabolites including alkaloids, phenols, terpenoids, saponins,flavonoids, tannins, glycosides, steroids. The quantitative estimation of crude bark extract shows the flavonoid content 24.81µg /ml. Yadav (2010) isolated flavonoid compounds from casuarina equiactifolia. The structural charactarisation of bioactive compounds was elucidating from methanolic bark extract [14]. Similar to Aherand Yadav work the bark extract of *Tamarindus indica* also showed significant amount of flavonoid content. In phosphomolybdenum reduction assay is based on the reduction of phosphomolybdateion in the presence of an antioxidant resulting in the formation of a green phosphate complex which is measured spectrophotometrically. The nitric oxide scavenging activity of flavonoids and phenolic compounds are known [15]. We can speculate that these constituents might be responsible for the observed nitric oxide scavenging activity.

Conclusion

The result of the present study showed that the bark extract of *Tamarindus indica* contain highest amount of flavonoid compounds, which exhibited the greatest antioxidant activity. The tested methanolic bark extract showed various significant bio-active molecules. There is no doubt that the plant bark is the reservoir of potentially useful chemical compounds. Which serve as drugs; provide newer leads and clues for modern drug design.

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