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PHYTOCHEMICAL ANALYSIS AND BIO EVALUATION OF *TERMINALIA RACEMOSA* BARK

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ABSTRACT

Terminalia racemosa is a member of medicinally important family Combretaceae. Five Solvent extracts of bark of the tree were explored for their phytochemical, cytotoxic and antioxidant activities. These extracts were hexane, chloroform, DCM, acetone and methanol extract. Bark extract of the tree were rich in secondary metabolites like flavonoids, anthraquinone, tannin, terpenoid and steroids. Hexane, chloroform and DCM extracts showed significant cytotoxic activity against brine shrimp assay ranging from 77% to 100% at a higher dose of 200microgram/ml. All the extracts possessed high level of antioxidant activity suggesting the tree medicinally at par with its popular members like *Termialia arjuna* and *Terminalia bellerica.*

KEYWORDS: *Terminalia racemosa,* Combretceae, Phytochemical Cytotoxic activity, Antioxidant activity.

INTRODUCTION

Terminalia racemosa is a lesser known plant of Terminalia genus belonging to family Combretaceae. *Terminalia racemosa* is widely distributed in tropical and subtropical countries and the leaves of this plant has been used as a medicine for treating dermatitis and hepatitis in Asian countries.[1] Two very important plants of Terminalia genus are *Terminalia bellerica* and *Terminalia chebula* commonly known as harida and bahada. Fruits of these are found in a number of ayurvedic formulations. Triphala is one such formulation commonly used for constipation, blood impurity and eye problems.[2] Owing to the excessive collection of the fruits of the two species, natural revegetation is scanty and the species are becoming vulnerable.[3] Another important plant of this family is *Terminalia arjuna*, bark of which has

also been reported for a number of properties like anti bacterial, anti inflammatory and blood purification etc.[4] In the present study bark of the tree *Terminalia racemosa* was explored for phytochemical, antioxidant and cytotoxic activity. Results have been discussed.

MATERIALS AND METHODS

Collection and Processing of plant material

The bark of medicinal plant *Terminalia racemosa* was collected from Botanical garden of Regional Plant Resource Centre, Bhubaneswar. Bark was washed thoroughly under running tap water to remove dust and pealed using knife. Further, they were dried in shade at room temperature followed by complete drying at 50 degree Celsius for 2hours in BOD incubator. Finally they were pulverized in a grinder (Lexus make) and fine powder so obtained was used for making solvent extracts.

Phytochemical analysis of bark of Terminalia racemosa

Phytochemical tests

Phytochemical test was conducted using standard protocols.[5]

Test for phlobotannin: Fresh bark powder of *Terminalia racemosa were* grounded with distilled water to make a solution. Then the mixture was filtered & the filtrate was taken as the sample. 1 ml of aqueous 1% HCl was added to the 1 ml of sample followed by boiling. A red precipitate is indicative of presence of phlobotanins.

Test for alkaloids: 1 ml of methanolic extract *was* filtered. Then 2 ml of 1% aqueous HCl was added to it. Then it was heated for few minutes. 2 drops of dragondroff reagent was added to the solution. Reddish brown precipitate with turbidity depicts alkaloids presence.

Test for flavonoids: To 5 ml of methanolic extract, 1 ml of 10% NaOH solution was added. From the side of the beaker 2 drops of concentrated HCl was added. Yellow colour turning to colourless is an indication of presence of flavonoids.

Test for anthraquinone: To 1 ml of methanolic extract, 2 ml of 5% KOH was added. Then the solution was filtered. Change in colour was observed. Pink colour shows the presence of anthraquinones.

Test for saponins: About 2 ml of 1% sodium bicarbonate was added to 1 ml of methanolic bark extract and shaked. Lather like formation persistent for some time is indicative of presence of Saponins.

Test for steroids: 100 µl methanolic extract of *T.racemosa* bark was taken in a test tube and 400 µl of acetic anhydride was added to it. Then 1-2 drops of concentrated sulphuric acid was added to it. Brown ring at the boundary of mixture shows the presence of steroids. (N.B. Test tube was kept in ice as exo thermic reaction occurs.)

Test for glycosides: 100 µl methanolic extract of *T.racemosa* bark was taken in a test tube and 400 µl of acetic anhydride was added to it. Then 1-2 drops of concentrated sulphuric acid was added to it. Blue-Green colour shows the presence of glycosides.

Test for tannin*:* 1gm of sample added with 100ml of distilled water, boiled and cooled, and then filtered. 1% ferric chloride was added drop wise to the filtrate. Green black precipitate shows the presence of tannin.

Test for terpenoid: 400 µl Chloroform was added to 1 ml of methanolic extract. Then 2-3 drops of sulphuric acid was added. Reddish/Brown colour shows the presence of terpenoid**.**

Test for starch: 1gm of dried powder was taken and grinded thoroughly using mortar pestle with 20ml of distilled water and filtered. Alcoholic iodine solution was added to the filtrate. Blue colour indicates the presence of starch.

Preparation of solvent extracts

Extracts were prepared using soxhlet apparatus as per standard protocols.[6] 15gms bark powder was subjected to extraction with 150 ml of hexane, Reflux was continued till the solvent became colourless. Extract obtained was concentrated in Buchhi Rotavapour. Concentrated extract was stored in screw cap vials until further use. Same process was repeated by other solvents like DCM, Chloroform, acetone and methanol.

Biological tests

Biological evaluation of all the extracts were done using two bench top models which were as follows:-

a) Cytotoxic activity using brine shrimp lethality assay.[7]
b) Antioxidant activity using DPPH assay.[8]

Brine shrimp lethality test

Brine shrimp (*Artemia Salina*) eggs were incubated for 48hrs (1.8gm of black salt in 100ml of distilled water) to get the desired growth of the larvae for biological evaluation. Stock solution of different extracts was prepared at a concentration of 20µg/ml. Extract was evaluated at four doses 25, 50,100 and 200 µg/ml. For each dose level three replicates were used. Motility, readings were taken every hour up to 4hours. Motility was graded as below:

4+ highly motile

3+ motile

2+ sluggish

1+ slow

Nil no activity at all

After 24hrs the final reading was taken and percentage of inhibition was calculated by comparing the treated samples with the controls. Standard deviation was also calculated.

Antioxidant activity

DPPH Assay

To detect antioxidant activity, qualitative 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assay was carried out. The plates were first air dried and then the chromatograms were sprayed with 0.2% 2, 2, diphenyl-1-picryl-hydrazyl in methanol as an indicator. The presences of antioxidant (AH) compounds were detected by yellow spots against a purple background on the TLC plates sprayed with 0.2%DPPH in methanol.

$DPPH + AH \rightarrow DPPH - H + A^{-}$

(Purple colour) (Yellow colour)

Qualitative screening of the constituents in each of the bark crude extracts of *T. racemosa for* antioxidant activity was done by TLC analysis. The process was carried out using TLC sheets. For about 5µl of each sample was loaded on the TLC sheet and the chromatograms were developed in following solvent systems:

a) Ethyl acetate: Methanol: Water (40:5.4:4) [EMW] (polar neutral)
b) Chloroform: Ethyl acetate: Formic acid (5:4:1) [CEF] (Intermediate polarity/acidic)
c) Benzene: Ethanol: Ammonium hydroxide (90:10:1) [BEA] (Non polar/basic)

RESULTS AND DISCUSSIONS

Five extracts of *Terminalia racemosa* bark were prepared, yield of DCM extract was highest with 3.02 gms followed by chloroform 2.624gms. Acetone extract showed a moderate yield of 1.4 gms where as yield of hexane and methanol extract was less than 1 gm.

Phytochemical analysis of bark of Terminalia racemosa

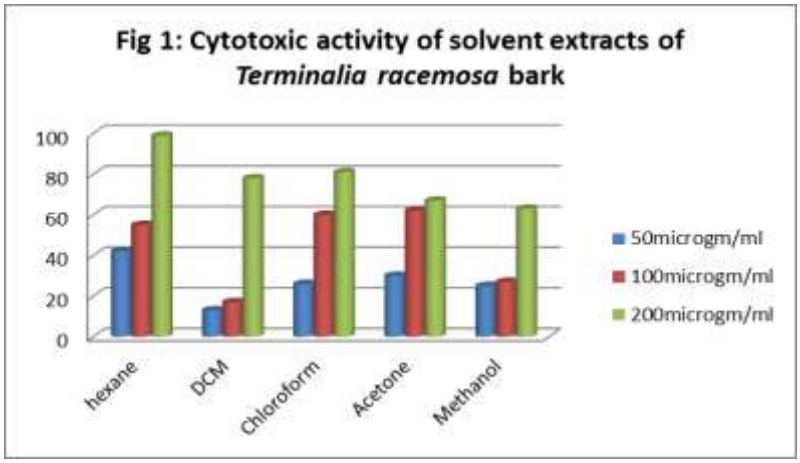
Crude methanolic extract was used for phytochemical analysis, as can be seen from Table 1, very significant metabolites of medicinal potential like Flavonoids, Anthraquinone, tannins and terpenoids were present in the extract. Study is in confirmation with previous studies[9] where terpenoids and tannins have been reported from a number of species of Terminalia genus and a number of molecules have been isolated as well.

Table 1: Phytochemical analysis of methanolic extract of *Terminalia racemosa* bark.

Phytochemical	Status
Phlobatannin	-ve
Alkaloid	-ve
Flavonoid	+ve
Anthraquinone	+ve
Saponin	-ve
Steroid	+ve
Glycoside	-ve
Tannin	+ve
Terpenoid	+ve

Cytotoxic activity of bark extracts of Terminalia racemosa

For cytotoxic activity motility of brine shrimps was observed for 4 hours and compared with that of controlled samples. It was observed that up to four hours there was no effect on the larvae, their motility was comparable with the controlled samples. But after 24 hrs in the samples, number of live larvae in each of the experimental tube was counted and percentage inhibition was calculated by comparing it with the positive controls. Activity was found to be dose dependent.



Antioxidant activity of terminalia racemosa bark extracts

As per the DPPH assay, all the solvents showed yellow bands showing the presence of antioxidant molecules in the extracts. As can be seen from Table 2 all the extracts exhibited antioxidant molecules in all the solvents. Maximum number of antioxidant band were 2 to 3 in extracts except in case of hexane in EMW solvent where there was no separation. In case of acetone and methanol extracts streak was obtained which suggest very high number of bands situated very close together so that it is difficult to count the number of antioxidant molecules in such samples. Finally it can be concluded that tree species like its other members is full of medicinal potential.

Table 2: TLC based antioxidant activity of solvent extracts of *Terminalia racemosa.*

Solvent extracts → Solvents ⇩	Hexane	DCM	Chloroform	Acetone	Methanol
Chloroform: ethyl acetate: formic acid (5:4:1) [CEF]	0.30, 0.99	0.99	0.31, 0.99	streak	streak
Benzene: ethanol: ammonium hydroxide (90:10:1)[BEA]	0.22, 0.35, 0.99	0.18, 0.35	0.22, 0.32	Streak from 0 - 0.3	Streak from 0 – 0.32
Ethyl acetate: methanol: water (40:5.4:4)[EMW]	Nil	0.45, 0.67	0.4	0.47	0.45

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