ABSTRACT

Fruit powder was subjected to serial solvent extraction using Soxhlet apparatus starting from hexane to methanol. All the five extracts namely hexane, DCM, chloroform, acetone and methanol were subjected to cytotoxic activity using brine shrimp assay at doses of 20, 50,100 and 200µg/ml. Dose dependent activity was recorded and highest activity was exhibited by DCM extract (100% mortality) followed by chloroform extract, which resulted in the mortality of 83% of brine shrimp larvae at the dose of 200µg/ml. TLC based antioxidant assessment revealed a number of antioxidant bands in all the extracts with maximum number of bands in DCM extract. Thus, from both the assays it can be concluded that DCM extract was the most potent fruit extract of *Terminalia racemosa*. Phytochemical analysis revealed the presence of tannin, saponin and terpenoids.

Key words  *Terminal racemosa*, antioxidant, cytotoxic activity, fruit extract

INTRODUCTION

Combretaceae family consists of two medicinally important genera namely *Terminalia* and *Combretum*, with a large number of species used in the Indian, Unani and Siddha system of medicines. The bark of *Terminalia arjuna* and *Terminalia bellerrica* is used medicinally against many deadly diseases. Fruits of both of the above are part of triphala a well known ayurvedic formulation. Fruits of *Terminalia arjuna* have been reported for their anti hyperglycaemic activities. Fruits of another species *Terminalia chebula* have shown cytoprotective effect against oxidant stress. Thus, it can be observed that fruits of *Terminalia* species has significant amount of medicinal properties, thereby making the fruits of lesser known species, *Terminalia racemosa* an interesting subject for experimental studies.

MATERIALS AND METHODS

Collection and processing of plant material: The fruits of medicinal plant *Terminalia racemosa* were collected from Botanical garden of Regional Plant Resource Centre, Bhubaneswar. Fruits were washed thoroughly under running tap water to remove dust and pulp was separated from seeds using a sharp knife. Further, they were dried in shade at room temperature. Finally they were pulverized in a grinder (Lexus make) to make a fine powder which was used for making solvent extracts for phytochemical analysis and bioassays.

Phytochemical Tests: One gm of powdered fruit material was macerated with 10 ml of methanol and filtered, filtrate was used for all the phytochemical tests except for starch test where maceration of fruit powder was done in distilled water. All the phytochemical tests were conducted as per standard protocols.

Test for alkaloids: sample was added to diluted hydrochloric acid and mixture was heated for few minutes. 2 drops of dragondroff reagent was added to the solution. Reddish brown precipitate with turbidity depicts the presence of alkaloids.

Test for flavonoids: 1 ml of extract was added to 1 ml of 10% NaOH solution. From the side of the beaker 2 drops of concentrated HCl was poured. Yellow color turning to colorless is an indication of presence of flavonoids.

Test for anthraquinone: To 2 ml of 5% KOH 1 ml of extract was added. Then the solution was filtered. Solution converts to yellow suggest the presence of anthraquinone.

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

Test for steroids and glycosides: Methanolic extract of *T. racemosa* fruit was taken in a test tube & 2 drops 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 where as blue green color shows the presence of glycosides.

Test for tannin: Fresh fruit sample was collected and aqueous extract was boiled & filtered. FeCl3 was added drop wise to the filtrate. Green Black precipitate shows the presence of tannin.

Test for terpenoid: Chloroform was added to 1 ml of fruit extract. Then 2-3 drops of sulphuric acid was added. Reddish/Brown color shows the presence of terpenoid.

Test for starch: 1gm of dried powder was taken and grinded thoroughly using mortar pestle with distilled water and filtered. Alcoholic iodine solution was added to it. Blue color shows the presence of starch.

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Preparation of Extracts for bioassays: The powdered plant material was subjected to serial solvent extraction using Soxhlet extraction assembly.

Biological evaluation: Biological evaluation of solvent extracts was conducted using two bench top models which were as follows:

1. Cytotoxic activity using brine shrimp lethality assay

2. Antioxidant activity
   a) Qualitative TLC based DPPH assay
   b) Quantitative DPPH radical scavenging assay
   c) Nitric oxide radical scavenging assay

Brine shrimp lethality test: Assay was conducted as per the standard protocols. 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 number of surviving larvae was counted in controls as well as experimental samples, percentage inhibition was calculated by comparing the treated samples with the controls. Standard deviation was also calculated.

Antioxidant activity: a)Thin layered chromatography based Qualitative antioxidant assay (DPPH ASSAY)

To detect antioxidant activity, qualitative 2, 2 diphenyl-1-picyrrylhydrazyl (DPPH) assay was carried out. All the extracts were run in three solvents as per the protocols of Eloff et al.

Solvents used were as follows:

1. ethyl acetate/methanol/water (40:5.4:4) [EMW]
2. chloroform/ethylacetate/formicacid(5:4:1) [CEF]
b) Quantitative DPPH radical scavenging assay: Radical scavenging DPPH assay were conducted using the standard protocols. 1.0mM solution of DPPH in methanol was prepared and 500µl of this solution was added to 4.0ml of extract solution (hexane, DCM, chloroform, acetone & methanol) in methanol at different concentrations (9.8 - 1250μg / ml). After 30 minutes incubation, the absorbance was measured at 517nm. Ascorbic acid at various concentrations (9.8 - 1250μg / ml) was used as reference compound. Lower the absorbance of reaction mixture indicates higher free radical scavenging activity. The capability to scavenge DPPH free radical was calculated using the following equation.

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\text{Percentage of Inhibition (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100
\]

Where \( A_{control} \) is the absorbance of control reaction and \( A_{test} \) is the absorbance of sample extracts. The antioxidant activity of the leaf extract was expressed as IC\(_{50}\) and compared with standard. The IC\(_{50}\) value was defined as the concentration (in µg/ml) of extracts that scavenges the DPPH radicals by 50%. IC50 of the extracts was compared with the standard antioxidant ascorbic acid.

c) Nitric oxide radical scavenging assay: Same was conducted using the standard protocol. 10mm nitroprusside in phosphate buffer saline is added to the test samples and incubated at 25 degree celsius for 150 minutes. After incubation test sample and griss reagent are mixed in the ratio of 1:1. Readings are taken at 546nm. Quercetin is used as standard.

RESULTS AND DISCUSSIONS

Phytochemical tests Terminalia racemosa fruit was found to be positive for saponins, tannin, terpenoids and steroids, remaining compounds namely anthraquinone, alkaloids, flavonoids and phlobotannins were absent. Pentacyclic terpenoids have also been reported from Terminalia superba. Terpenoids are generally found in fruits and are considered to be natural antioxidants. Similarly saponins are considered as pharmacological active molecules and have been reported to be good in cytotoxic activity and a number of oleanolic saponins are being explored for anticancer activities. Similarly steroids such as withanolides from Withania somnifera have significant activity against tumor necrosis factors. Thus all the phytochemical found in the fruit extracts of Terminalia racemosa indicate towards the biologically active potential of the fruits of the species.

Cytotoxic activity: Cytotoxic activity motility of brine shrimps was observed up to 4 hours and their mortality was observed after 24 hrs and compared with that of controls. As shown in Table 1, 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, a dose dependent mortality was observed. DCM extract showed the highest amount of cytotoxic activity followed by chloroform extract. Earlier also bark extract of Terminalia arjuna has depicted cytotoxic activity. Fruit extract of Terminalia chebula has also shown cytotoxic potential against skin fibroblast. Thus, fruit extracts of Terminalia racemosa like other members of the same genus has shown cytotoxic potential. Hydrolysable tannins were found to be responsible for cytotoxic potential of Terminalia chebula fruits. Tannins were also present in the fruits of Terminalia superba.
racemosa, which could be responsible for the cytotoxic activity of the plant.

Antioxidant activity

In TLC base DPPH assay, all the solvents showed yellow bands confirming the presence of antioxidant molecules in the extract. As can be seen from Table 2, all the extracts showed more than one antioxidant bands suggesting them to be positive for antioxidant potential. In quantitative assays as can be seen in both the assays(Fig 1 and 2), Acetone extract showed highly significant antioxidant activity in both the assays DPPH radical scavenging assay and Nitric oxide reduction assays. This study is in confirmation with the other studies in which a number of fruits of Terminalia species has shown good antioxidant potential\(^5\,\!^6\). All the above results indicate towards the medicinal importance of fruits of Terminalia racemosa and a detailed investigation in the form of bioassay guided isolation of molecules is warranted. Keeping in view of the antioxidant potential, fruits of Terminalia racemosa could also be tried in preparation of Ayurvedic formulations.

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