Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2013; 5(3); 151-154

ISSN: 0975-4873

Research Article

Phytochemical Analysis and Evaluation of Cytotoxic and Antioxidant Activity of Fruit Extracts of *Terminalia Racemosa*

*Bhatnagar S, Panda P, Behera DR

Medicinal and Aromatic Plants, Regional Plant Resource Centre, Nayapalli, Bhubaneswar, India

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 *Terminalia 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 bellerica* is used medicinally against many deadly diseases. Fruits of both of the above are part of triphala a well known ayurvedic formulation[1]. Fruits of *Terminalia arjuna* have been reported for their anti hyperglycaemic activities[2]. Fruits of another species *Terminalia chebula* have shown cytoprotective effect against oxidant stress[3]. 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[4].

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.

*Author for correspondence: E-mail: bhasunita@gmail.com

Table 1. TLC based DPPH assay

Solvent extract / Solvent	Hexane	DCM	Chloroform	Acetone	Methanol
Benzene/ethanol/ammonium hydroxide (90:10:1)[BEA]	5	3	3	Infinite	Infinite
Chloroform/ethylacetate/formicacid(5:4:1)[CEF]	4	7	4	1	1
Ethyl acetate/methanol/water (40:5.4:4)[EMW]	4	4	2	Infinite	Infinite

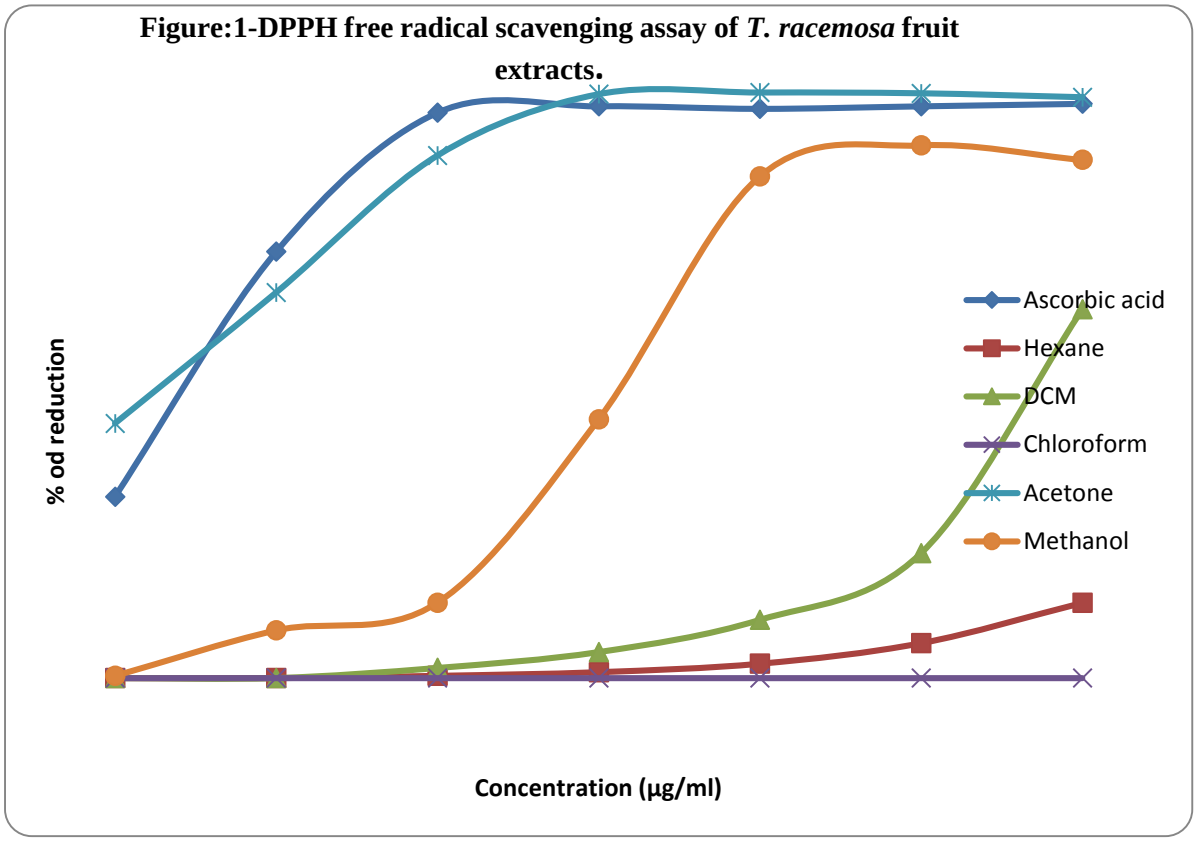


Figure:1-DPPH free radical scavenging assay of *T. racemosa* fruit extracts.

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[5]
2. Antioxidant activity

a) Qualitative TLC based DPPH assay[6]
b) Quantitiative DPPH radical scavenging assay[7]
c) Nitric oxide radical scavenging assay[8]

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-picrylhydrazyl (DPPH) assay was carried out. All the extracts were run in three solvents as per the protocols of Eloff et al [6].

Solvents used were as follows:

1) ethyl acetate/methanol/water (40:5.4:4)[EMW]
2) chloroform/ethylacetate/formicacid(5:4:1)[CEF]

3) benzene/ethanol/ammonium hydroxide (90:10:1)[BEA] 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 compounds were detected by yellow spots against a purple background on the TLC plates

DPPH + AH → DPPH – H + A^-

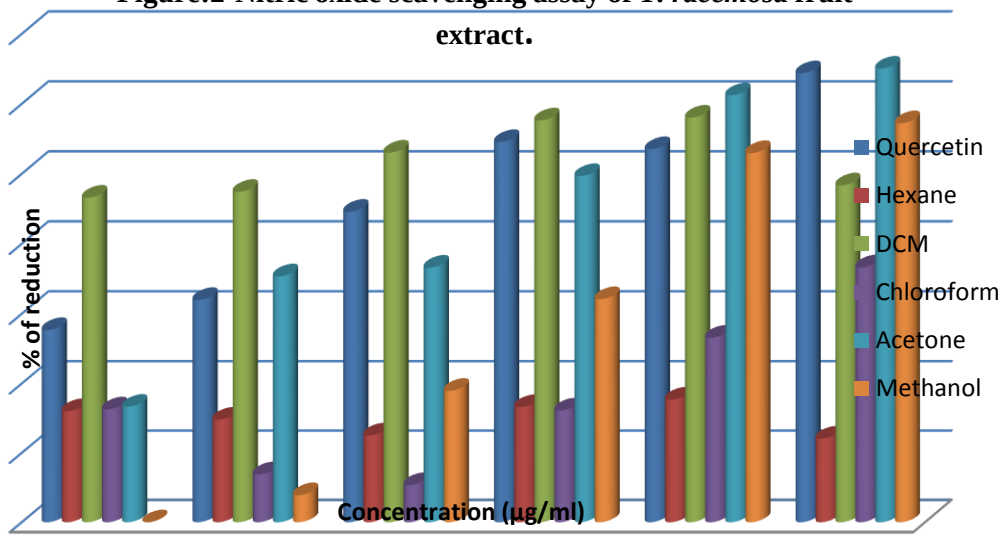
(Purple colour) (Yellow colour)

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

Figure:2-Nitric oxide scavenging assay of *T. racemosa* fruit extract.



b) Quantitative DPPH radical scavenging assay*:* Radical scavenging DPPH assay were conducted using the standard protocols[7]. 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.

Percentage of Inhibition (%) = *Acontrol – Atest / Acontrol X 100*

Where *Acontrol* 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[8]. 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

terpenoids have also been reported from *Terminalia superba.* Terpenoids are generally found in fruits and are considered to be natural antioxidants[9]. 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[10]. Similarly steroids such as withanolides from *Withania somnifera* have significant activity against tumor necrosis factors[11]. 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[12], Fruit extract of *Terminalia chebula* has also shown cytotoxic potential against skin fibroblast[13]. 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[14]. Tannins were also present in the fruits of *Terminalia*

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[15,16]. 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.

ACKNOWLEDGEMENT

Acknowledgements are due to Dr. P.C.Panda for taxonomic guidance. Authors wish to acknowledge the support of Forest and Environment, Govt. of Orissa for providing laboratory facilities.

REFERENCES

1. Kumar AD and Prasad MVV. Antimicrobial activity of Triphala churnam. *Aryavaidyan 2004***;** 18(2): 109-111**.**
2. Murali YK., Chander C, and Murthy PS. Anti hyperglycemic effect of water extract of dry fruits of *Terminalia chebula* in experimental diabetes mellitus. *Ind J Clin Biochem* 2004. 19, 202-204.
3. Na MK, Bae KH, Kang SS, Min BS, Yoo JK, Kamiryo Y, Senoo Y, Yokoo SM Cytoprotective effect on oxidative stress and inhibitory effect on cellular aging of Terminalia chebula fruit. *Phytotherapy research* 2004; 19 (9): 737-741.
4. Saraswathy A, Amla K, Shakila R, Kumar KNS. Pharmacognostic and preliminary phytochemical studies on the stem of *Terminalia alata* Heyne ex. Roth. *Journal of Medicinal and Aromatic plant science* 2009; 31(1); 42-47**.**
5. Bhatnagar, S and Pattanaik, SR. Comparative analysis of cytotoxic and antioxidant activities of leaf and bark extracts of *Clerodendrum viscosum* and *Clerodendrum phlomidis. Int J Biomed Adv Res* 2012; 4: 231-239.
6. Eloff JN, Katrere D, Mc Gaw LJ. The biological activity and chemistry of the Southern African Combretaceae. *Journal of Ethnopharmacology* 2008; 119 (3): 686-699.
7. Deby, C and Margotteaux G. Relationship between essential fatty acids and tissue antioxidant levels in mice. *C R Seances Soc Biol fil* 1970; 165: 2675-2681.
8. Panda BR, Mohanta SR, Manna AK, and Si S. *In vitro* antioxidant activity of the aerial parts of *Cocculus hirsutus*, Diels. *J Adv Pharm Res* 2011; 2(1): 18-23.
9. Tabopda TK, Ngoupasp J, Tanoll SAK, Mitaine AC, Ngadjul BT, Ali MS, Luu, B.; Lacaille-DMA. Antimicrobial pentacyclic triterpenoids from *Terminalia subera*. *Planta medica* 2009; 75(5):522-527
10. Antoaneta I, Juliana S, Dragomir D, Imre O, Ivanka K and Molnar J. Screening of some saponins and phenolics components of *Tribulus terrestris* and *Smilax excels* as MDR modulators. *In Vivo* 2009; 23: 545-550.
11. Haq IL, Youn UJ, Chai X, Park EJ, Kondratyuk TP, Simmons CJ, Borris RP, Mirza B, Pezzuto JM and Chang LC. Biologically active withanolides from Withania coagulans. *J Nat Prod*. 2013; 76: 22-28.
12. Halder S, Bharal N, Mediratta PK, Kaur IS, Sharma, KK Antiinflamatory, immuno modulatory antinociceptive activity of *Terminalia arjuna* Roxb. Bark in mice and rats .*Indian Journal of Expermental Biology* 2009; 47(7) : 577-583
13. Khwunjit I, Kornkanok I, Malyn C and Jarupa V. Antioxidant activity and cytotoxicity to human skin fibroblasts of Terminalia chebula fruit extract. *2012 International Conference on Biological and Life Scienc IPCBEE vol.40 IACSIT Press, Singapore*
14. Seung HL, Shi YR, Sang UC, Chong OL, Zaesung NO, Siong KK, Jhong WA. Hydrolysable tannins and related compound having cytotoxic activity from fruits of Terminalia chebula. 1995; 18: 118-120.
15. Chander, R, Khanna AK, Mathur SK, Kar A, Rastogi M. Antidyslipemic and antioxidant activities of SBC; A herbal preparation. *Ethnobotany* 2002; 14(1and2): 123-128.
16. Patel NM, Patel JL, Patel PM, Patel VP, Bhavsar, GC. Hepatoprotective activity of *Terminallia bellerica* in correlation with its significant antioxidant property. *Journal of phyto pharmaco therapy and Natural products2008*; 1(1) :37-48