Original Article

Comparative Study of Green Fruit Extract of *Melia azedarach* Linn. With its Ripe Fruit Extract for Antileishmanial, Larvicidal, Antioxidant and Cytotoxic Activity

Imran Khan[*1], Muhammad Masoom Yasinzai[1], Zaffar Mehmood[1], Ikram Ilahi[2], Jangrez Khan[3], Ali Talha Khalil[1], Muhammad Shahab Saqib[1] and Waheed Ur Rahman[4]

[1]Department of Biotechnology, Quaid-i-Azam University Islamabad, Pakistan-45320
[2]Department of Zoology, University of Malakand, Pakistan-24800
[3]Department of Biochemistry, Quaid-i-Azam University Islamabad, Pakistan-45320
[4]Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan

Address for Correspondence
Department of Biotechnology, Quaid-i-Azam University Islamabad, Pakistan-45320.

E-mail: imrankhan572@yahoo.com

ABSTRACT

The present study was carried out to compare the potential of green and ripen fruits aqueous extract against antileishmanial, larvicidal, antioxidant and brine shrimp cytotoxicity assay. A general antileishmanial, larvicidal, antioxidant and cytotoxic assays were performed at different concentrations. The green fruits expressed significant activity against *L. tropica* (LC_{50}=0.41µg/mL), while the ripe fruits showed less activity (LC_{50}=8227.51µg/mL). Upon larvicidal activity against *Culex quinquefasciatus* larvae, the green fruits extract expressed excellent results with LC_{50} value 67.65µg/mL while ripe fruits showed less activity with LC_{50} value 7171.18µg/mL. Similarly, for cytotoxicity assay for green and ripe fruits the LC_{50} value obtained were 18.07µg/mL and 530.2µg/mL, respectively. The result for antioxidant potential showed that only the green fruits has some antioxidant potential (IC_{50} 232.23µg/mL) compared to ripe fruits (>1000µg/mL) against the reference drug (ascorbic acid). The total phenolics contents of the green fruits expressed good concentration 10.54 mg/g DW while ripe fruits have 5.32 mg/g DW. From these results it can be concluded that green fruits has more active compounds than ripe fruits. The green plant material can be used as good source of antiprotozoal, insecticidal and anti-cancerous candidate.

Keywords: Antioxidant, Brine shrimps, *Culex quinquefasciatus, Leishamania tropica*, *Melia azedarach*.

INTRODUCTION

Traditional medicine is used all over the world because of the availability of plant species and plant derived products[1]. The extensive use of traditional medicine could be accredited to economic affordability, cultural acceptability, and efficacy against different type of diseases as compared to current medicines. Thus, all around the world different local communities have indigenous knowledge in different medicinal plants where they exercise their skills and perceptions to classify plants and their parts to be used for various ailments[2,3].

New approaches have been adopted by the scientific community for the development of new medicine. The emerging trend in resistance development can be attributed to the indiscriminate uses of drugs for the treatment of infectious diseases[4]. The scientists throughout the world now eager to search for new drugs from different sources including medicinal plants. So far, about 25-45% of the modern prescriptions include plant based molecules as an active ingredient in drug formulations[5,6].

Pakistan is blessed with extravagant medicinal plants species including *Melia azedarach Linn. M. azedarach* is a perennial tree found across sub Himalayan belt belongs to family Meliacea which constitutes 45 genus and more than 750 species[7]. *M. azedarach* is locally known as "Thora shandai" in Mardan Khyber Pakhtunkhwa, Pakistan and its common names includes Persian Lilac, Bakain and China berry. Traditionally, *M. azedarach* is used to treat a range of ailments such as antimalarial, antibacterial, antiviral, anti fertility and anticancer[8-10]. The extract obtained from different parts of the plant used for febrifuge, stomach ache, cystitis, intestinal disorders, uterine illnesses, diabetes and diuretic has also been reported[11,12]. Different parts of the plant are used for different ailments. The fruits extract of the plant elicit numerous effects in insects like growth retardation, antifeedent, reduce fecundity, morphogenetic defect, molting and changes of behavior[13-17]. We have previously reported the larvicidal activity of aqueous extract of ripe fruits of *M. azedarach* against *Culex quinquefasciatus*[18].

To best of our knowledge, there is no activity has been reported on aqueous extract of green fruits of *M. azedarach*. The present study was design to evaluate the hidden potential of green fruits with comparison of ripe fruits for different pharmacological activities such as antileishmanial, antioxidant, larvicidal and cytotoxic activities.

MATERIALS AND METHODS

Collection and identification of Plant

Plant materials (green and ripe fruits) were collected from the vicinity of Quaid-i-Azam University, Islamabad, Pakistan in June and November 2013. The plant was then identified by a trained botanist Prof. Dr. Zabta Khan Shinwari, Professor, Department of Biotechnology, Quaid-i-Azam University Islamabad. A voucher specimen was then deposited to the herbarium. The fruits were washed with distilled water and stored in dry place till process.

Extraction

About 1 kg each of fruits was macerated by mechanical grinder. The material was soaked in 5 liter plastic beaker in distilled water for 6 days with occasional shaking. After 6 days, the plant material was then filtered two times through a muslin cloth. The filtrate was then subjected to rotary evaporator (Heidolph Laborta4000 efficient) and dried under reduce pressure.

The extract obtained was stored at 4°C for further use.

Total phenolics contents (TPC) determination

Chemicals and materials used

Folin-Ciocalteu reagent, Sodium carbonate, Galic acid, ice bath, water bath, UV-Vis spectrophotometer (Shimadzu-1700).

Procedure

TPC was determined according to the procedure previously described by Ali *et al*. (2013) with slight modification[19]. The absorption of test samples was measured at 725 nm by using UV-Vis Spectrophotometer (Shemadzu-1700). The TPC was shown as Gallic acid equivalent per gram of the dry weight.

In vitro Antileishmanial Assay

Chemicals and materials used

Medium 199, Fetal Bovine Serum, 96 well plate, Amphotericin B, Dimethyl sulfoxide, micro pipettes, Neubauer counting chamber, Light microscope and incubator.

Culture of Parasites

L. tropica kwh 23 strain was kindly gifted by Prof. Dr. Akram Shah (Professor at Department of Zoology, University of Peshawar, Khyber Pakhtunkhwa Pakistan). The strain was incubated at 24±1°C for 6-7 days in 199 medium containing 10% Fetal Bovine Serum.

Samples Preparation

The *In vitro* antileishmanial assay was performed according to the protocol previously described by Nabi *et al*. (2012) with a slight modification[20]. Stock solution (10,000µg/mL) of the samples were prepared by dissolving 10 mg in 1000 µL of distilled water. The stock solutions were serially diluted in 96 well plates. Positive and negative control was maintained having Amphotericin B and distilled water respectively. The microtitre plates were incubated at 24°C for 72 hrs. The experiment was performed in triplicate. After 72 hrs, about 15 µl of test culture were then transferred to improved Neubauer counting chamber and live promastigotes were counted under light microscope. For LC_{50} calculation Probit regression Analysis of SPSS Ver. 21 was used.

Larvicidal bioassay

Chemicals and materials used

Plastic jar, Dimethyl sulfoxide, net cloth, dog biscuits, incubator, permithrin and needles.

Test organism

Mosquito larvae of *C. quinquefasciatus* were kindly gifted by Mr. Ikram Ilahi, Department of Zoology, University of Malakand, Chakdara Pakistan.

Experimental procedure

The larvicidal assay was carried out according to the procedure describe by Ilahi *et al*. (2012)[18]. Briefly, the mosquitoes larvae were reared in the laboratory in plastic jar covered with net cloth. The larvae were fed by dog biscuits and brewer's yeast in 2:3 ratios. The stock solution (10000µg/mL) of the extracts was prepared. The activity was performed in concentration ranges from 20-2000µg/mL. A total of 20 3rd and 4th instars larvae were transferred to each 100 mL beaker containing plant extract. A positive and negative control was maintained containing Permithrin 0.3% and distilled water respectively. The beakers were kept in standard laboratory conditions i.e. 30 ± 2°C and 70-75% relative humidity.

After 24 h, the dead larvae were counted when it fails to move by touching with needle at cervical or siphon region of the larvae. The experiment was performed in triplicate.

Antioxidant Activity

Chemicals and materials used

Methanol (Analytical grade), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (sigma Aldrich co.) DMSO (RDH), Ascorbic acid (Sigma Aldrich).

Sample preparation

For 10, 000µg/mL stock solution, 10 mg of the test samples dissolved in1mL of DMSO. The stock solutions were then diluted to get a final concentration of 20, 40, 60, 80 and 100µg/mL.

DPPH free radical scavenging assay

DPPH free radical scavenging assay of the test sample and standard was accessed as described previously by Ilahi *et al*. (2013) with a slight modification[21]. Briefly, DPPH (0.004%) was dissolved in methanol. The experiment was carried out by dissolving 1 mL of DPPH solution in 1 mL of the test sample. The solution mixture was incubated for half an hour in dark area of the lab. For blank 1 mL of methanol plus 1 mL of DPPH was used, Ascorbic acid was used as standard. After specified time the absorption of the test compounds were measured at 517 nm on UV-Vis spectrophotometer (Shimadzu-1700). The % absorption was then calculated by the formula given below:

% inhibition = A-B/A X 100.

Where A = absorption of blank, B = absorption of test sample.

Cytotoxicity assay

Chemicals and materials used

The materials used were: tray (for hatching eggs), lamp (for larvae attraction), micro pipette, sea salt (4% distilled water, pH 7.4), test sample, Dimethyl sulfoxide (DMSO) (Sigma Aldrich).

Sample preparation

In vitro brine shrimp cytotoxicity assay was carried out according to the protocol previously describe by Ali *et al*. (2011) with a slight modification[22]. Exact 10 mg of test sample was dissolved in 1mL of DMSO. Briefly, the experiment was performed at different concentration ranging from 2 to 1000µg/mL. A total of 10 shrimps were transferred into each vials containing test samples by means of dropper. The final volume of the test samples were adjusted to 5 mL by sea salt. The assay was performed in triplicate. The vials were then incubated for 24 hrs at 25^{o}C. Sea salt containing DMSO was used as negative control; DMSO plus drug Doxorubicin was used as positive control. After 24 hrs of exposure the result obtained were then statistically analyzed by Probit regression analysis SPSS Ver. 21 software to get LC_{50} value.

Statistical analysis

The data obtained were subjected to SPSS Ver. 21 (IBM, New York, US). For LC_{50} value, Probit regression analysis test was performed. To compare the extract, Post Hoc Duncan Multiple Comparison test in One Way ANOVA was carried out. All the values are given mean± standard deviation. The Probability of $P<0.05$ considers as significant at 95% confidence interval.

RESULTS AND DISCUSSION

The *in vitro* screening of plants for its pharmacological assays has an advantage

of low cost and fast turn over which made the plant to be screened at large scale. Leishmaniasis is a neglected tropical disease with several clinical manifestations[23]. Upon antileishmanial potential of the plant extract, green fruits showed significant activity ($P<0.05$) when compared to the ripe fruits as shown in Table 1. The LC_{50} value for green and ripen fruits is 0.49 µg/mL and 8227.5 µg/mL, respectively. Amp B was used as reference drug with LC_{50} value 0.39µg/mL. The % survival of promastigotes is expressed in Figure 1. Due to lack of proper control, its incidence is raising worldwide. Besides that, the resistance of *Leishmania* against first line drug glucantime and also its long time use may cause renal and cardiac problems[24,25]. The existence of noxious problems that is resistance and absence of vaccines warn the scientific community for new therapy that protect against and/or treat leishmaniasis. However, *M. azedarach* exhibited anti-inflammatory immuno-modulatory, nematicidal, antihyperglycemic anticarcinogenic, insecticidal, antiviral, antiparasitic and antioxidant properties[26]. Alharmni *et al*. (2011) reported the in vivo effect of aqueous extract of ripen fruits on some biochemical parameters of infected mice with *L. donovani*. In their study, it was reported that the plant has elevated the level of ALT, AST while decline the level of LDH and ALP and there is non significance difference in the level of cholesterol, glucose and protein when compared to non treated group. In the same study, they suggested that fruits of *M. azedarach* could be a novel approach for combined drug therapy for visceral leishmaniasis[25]. In the present study the aqueous extract of green and ripen fruits were investigated for its antileishmanial potential. The increased activity of the green fruits may be attributed to higher amount of lemonoids and azadirachtin present or may have some new compounds to be isolated.

Similarly, the brine shrimp cytotoxicity result is summarized in Table 2. Green aqueous extract of the plant fruits showed significant activity when compared to the ripe fruits ($P<0.05$). The LC_{50} value for green and ripen fruits is 18.07 and 530.2µg/mL, respectively. For positive control Doxorubicin was used. The LC_{50} value for Doxorubicin is 5.93µg/mL. The % survival of the brine shrimps is expressed in Figure 2. The degree of cytotoxicity level was observed which depend on the concentration of the drug used. The mortality rate of brine shrimp is concentration dependent. As the concentration of the plant extract increases the % survival decreases[27]. The brine shrimp activity indicates cytotoxicity as well as leads to further pharmacological activities such as antitumor, antimicrobial, pesticidal, etc[28]. Zhou *et al*. (2005) reported the limonoids isolated from the ripe fruits exhibited inhibitory activity against HeLaS3 cancer cells[29]. The green fruits fraction of the plant was found to have excellent activity against brine shrimp.

The plant *M. azedarach* recognize for its insecticidal and medicinal properties. The fruits, although are the poisonous part of the plant but has also medicinal values[30]. The insecticidal potential of the plant is due the active compound lemonoids which include melianoninol, melianol, melianone, meliantriol, meliandiol, trichilins, Salannin, toosendanin, nimbin, meliacarpinin, salannal, lignanes and azadirachtin[31]. The larvicidal potential of the aqueous fruits extract is summarized in Table 3. The green fruits showed significant activity when compared to ripen fruits of the plant ($P<0.05$). The LC_{50} value for green fruits is 67.65µg/mL and 3047.6µg/mL for that of ripe fruits. The Table 3 indicates the LC_{90} value for both green and ripe fruits 142.92 and 7171.18µg/mL, respectively. Their % survival is shown in figure 3. These finding

is against the finding of the Ilahi *et al*. (2012) which showed the LC_{50} value of shade dried fruits was 2035.13µg/mL[18]. The augmented activity of green fruits may be due to the high content of the active ingredients or may be due to some novel compounds present in the aqueous extract which probably evaporates with the passage of time or may be converted to some other inactive form. However, the hexane fraction of fruits has strong larvicidal activity against malarial vector *Anopheles stephensi*[32].

Likewise, the result of the DPPH free radical scavenging assay in % inhibition is given in Figure 4. The result indicates that the test samples expressed no significant activity when compared to ascorbic acid

(Standard) ($P>0.05$). The maximum activity 42.59 ± 3.12 and 14.66 ± 1.42 was recorded for green and ripen fruits extract, respectively at highest concentration (100µg/mL), while at this concentration the ascorbic acid expressed significant activity 83.23 ± 1.68 ($P<0.05$). The IC_{50} values for green and ripe fruits are 232.23 and >1000µg/mL, respectively. The DPPH free radical scavenging provides a good model to investigate the antioxidant potential of a test compound in very short time and sensitive when compared to the other methods. The result indicated that the % inhibition of the plant extract is dose dependent. The study showed the green extract has the more proton donating capability than ripen fruits and may serves as free radical scavenger. Though the antioxidant activity of the Maleacea family attributed to the phenolics contents present in the extract. Our result is in contrast to *Munir et al.* (2012) finding, whose result showed 63.87 % activity for sun dried fruits[33]. The result for total phenolics contents indicates that green fruits exhibited good activity (10.45 mg/g DW) which is significantly higher than that of ripe fruits (5.32 mg/g DW). Munir *et al*. (2012) investigated the TPC in aqueous-methanol solvent extract of different parts of the *M. azedarach* plant. The result obtained indicated good phenolics contents in sun dried fruits extract (74.43mg/g DW) followed by shade dried fruits extract (66.89mg/g DW) which is also contrast to our findings[31]. The highest phenolics contents were previously reported in ethanolic fraction of the plant material. Nahak and Sahu, (2010) further reported that the highest antioxidant activity for ethanol followed by aqueous and methanolic extract of *M. azedarach* leave[34]. Thus it is clear that phenolics contents are concentrated in organic solvent and less in aqueous solvent.

CONCLUSION

Excellent results were shown by green fruits against all the activities i.e. antileishmanial, larvicidal and cytotoxic except mild activity was recorded for antioxidant potential. Therefore, further research is needed to isolate the active compounds and carry further analysis.

ACKNOWLEDGEMENT

The authors are thankful to Prof. Dr. Zabta Khan Shinwari (Chairman, Department of Biotechnology, Quaid-i-Azam University, Islamabad) for their encouragement and providing lab facilities.

Conflict of interest

The authors declare that they have no competing interests.

REFERENCES

1. Awas T, Demissew S, editors. Ethnobotanical study of medicinal plants in Kafficho people, southwestern Ethiopia. Proceedings of the 16th International Conference of Ethiopian Studies; 1999:

Trondheim. Institute of Biodiversity Conservation, Addis Aababa.
2. Omoruyi B, Bradley G, Afolayan A. Ethnomedicinal survey of medicinal plants used for the management of HIV/AIDS infection among local communities of Nkonkobe Municipality, Eastern Cape, South Africa. *Journal of Medicinal Plants Research*. 2012; 6:3603-8.
3. Shah SMH, Shah SMM, Nisar M, Khan FA, Ali M, Khan I, *et al*. Antimicrobial Activities of Medicinal Plants Used in Folk Remedies in Pakistan. *Journal of Pharmacy Research* Vol. 2012; 5(4):2057-60.
4. M AM, M M-O-RAN, M TN, K SM, S N. Pharmacological Potentials of *Melia azedarach* L. - A Review. *American Journal of Bioscience*. 2013; 1(2):44-9.
5. Cordell GA. Biodiversity and drug discovery-a symbiotic relationship. *Phytochemistry*. 2000; 55(6):463-80.
6. Ramya S, Jepachanderamohan P, Kalayanasundaram M, Jayakumararaj R. In vitro antibacterial prospective of crude leaf extracts of *Melia azedarach* Linn. Against selected bacterial strains. *Ethnobotanical Leaflets*. 2009; 2009(1):32.
7. A G. Chromophytes of Iran. 2nd ed. ed. Tehran: Tehran University Publication; 1994.
8. Watt J, Breyer-Brandwijk M. The Medicinal and Poisonous Plants of Southern and Eastern Africa (2nd edn.) Livingstone. Edinburgh, London. 1962:205-6.
9. Baquar SR. Medicinal and poisonous plants of Pakistan. Medicinal and poisonous plants of Pakistan. 1989.
10. Rana A, Vishnukanta. *Melia azedarach*: *A phytopharmacological review*. 2008 January 1, 2008; 2(3):173-9.
11. Perry LM, Metzger J. Medicinal plants of east and southeast Asia: attributed properties and uses: MIT press; 1980.
12. Ahmad M, Qureshi R, Arshad M, Khan MA, Zafar M. Traditional herbal remedies used for the treatment of diabetes from district Attock (Pakistan). *Pak J Bot*. 2009; 41(6):2777-82.
13. Wandscheer CB, Duque JE, da Silva MA, Fukuyama Y, Wohlke JL, Adelmann J, *et al*. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. *Toxicon*. 2004; 44(8):829-35.
14. Schmidt G, Rembold H, Ahmed AA, Breuer M. Effect of *Melia azedarach* fruit extract on juvenile hormone titer and protein content in the hemolymph of two species of noctuid lepidopteran larvae [insecta: Lepidoptera: Noctuidae]. *Phytoparasitica*. 1998; 26(4):283-91.
15. Hammad E, Zournajian H, Talhouk S. Efficacy of extracts of *Melia azedarach* L. callus, leaves and fruits against adults of the sweetpotato whitefly *Bemisia tabaci* (Hom., Aleyrodidae). *Journal of applied Entomology*. 2001; 125(8):483-8.
16. Gajmer T, Singh R, Saini R, Kalidhar S. Effect of methanolic extracts of neem (*Azadirachta indica* A. Juss) and bakain (*Melia azedarach* L) seeds on oviposition and egg hatching of Earias vittella (Fab.)(Lep, Noctuidae). *Journal of applied Entomology*. 2002; 126(5):238-43.
17. Banchio E, Valladares G, Defagó M, Palacios S, Carpinella C. Effects of *Melia azedarach*,(Meliaceae) fruit extracts on the leaf miner *Liriomyza huidobrensis*,(Diptera, Agromyzidae): Assessment in laboratory and field experiments. *Annals of Applied Biology*. 2003; 143(2):187-93.
18. Ilahi I, Khan I, Tariq M, Ahmad I. Larvicidal Activities of Different Parts of *Melia azedarach* Linn. Against *Culex quinquefasciatus* Say. (Diptera: Culicidae). *Journal of Basic & Applied Sciences*. 2012; 8:23-8.
19. M A, BH A, Ihsan-ul-haq. Production of commercially important secondary metabolites and antioxidant activity in cell suspension cultures of *Artemisia absinthium* L. *Industrial Crops and Products*. 2013; 49 400- 6.
20. Nabi S, Ahmed N, Khan MJ, Bazai Z, Yasinzai M, Al-Kahraman YM. In vitro Antileishmanial, Antitumor Activities and phytochemical Studies of Methanolic Extract and its Fractions of Juniperus Excelsa Berries. *World Applied Sciences Journal*. 2012; 19(10):1495-500.

21. Ilahi I, Samar S, Khan I, Ahmad I. In vitro antioxidant activities of four medicinal plants on the basis of DPPH free radical scavenging. *Pakistan journal of pharmaceutical sciences*. 2013; 26(5):949-52.
22. Niaz A, Ghayour A, Syed AS, Ismail S, Mehreen G, Imran K. Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of callistemon citrinus curtis. BMC Complementary and Alternative Medicine. 2011; 11.
23. Yasinzai M, Khan M, Nadhman A, Shahnaz G. Drug resistance in leishmaniasis: current drug-delivery systems and future perspectives. *Future medicinal chemistry*. 2013; 5(15):1877-88.
24. Carneiro SM, Carvalho FA, Santana L, Sousa AP, Chaves M. The cytotoxic and antileishmanial activity of extracts and fractions of leaves and fruits of *Azadirachta indica* (A Juss.). *Biological research*. 2012; 45(2):111-6.
25. Al-Harmni KIF, AL-Khan HI, Fattohy ZI. Effect of Aqueous Extract of *Melia azedarach* on the some Biochemical Parameters in the Liver Extract of Infected Mice by Lieshmania donovani.
26. Melo E, Vilela K, Carvalho C. Effects of aqueous leaf extracts of *Azadirachta indica* A. Juss.(neem) and *Melia azedarach* L.(Santa Barbara or cinnamon) on the intracellular development of Toxoplasma gondii. *Revista Brasileira de Plantas Medicinais*. 2011; 13(2):215-22.
27. Apu AS, Bhuyan SH, Khatun F, Liza MS, Matin M, Hossain MF. Assessment of cytotoxic activity of two medicinal plants using brine shrimp (*artemia salina*) as an experimental tool.
28. JE A, CJ C, JL M. Bioactive components of Allamanda nodiflora Linn. *Journal of Natural Products*. 1988; 51:307-8.
29. Zhou H, Hamazaki A, Fontana JD, Takahashi H, Wandscheer CB, Fukuyama Y. Cytotoxic limonoids from Brazilian *Melia azedarach*. *Chemical and pharmaceutical bulletin*. 2005; 53(10):1362-5.
30. Kim M, Kim S, Park B, Lee K, Min G, Seoh J, *et al*. Antiviral effects of 28-deacetylsendanin on herpes simplex virus-1 replication. *Antiviral research*. 1999; 43(2):103-12.
31. Li X. Recent studies on insecticidal activities of limonoids from meliaceous plants. *Insect Science*. 1999; 6(3):283-8.
32. Hadjiakhoondi A, Sadeghipour-Roodsari H, Vatandoost H, Khanavi M, Abai M, Vosoughi M, *et al*. Fatty acid composition and toxicity of *Melia azedarach* L. fruits against malaria vector *Anopheles stephensi*. *Iran J Pharm Sci*. 2006; 2(2):97-102.
33. Munir A, Sultana B, Babar T, Bashir A, Amjad M, Hassan Q. Investigation on the antioxidant activity of leaves, fruit and stem bark of Dhraik (*Melia azedarach*). *Eur J Appl Sci*. 2012; 4:47-51.
34. Nahak G, Sahu RK. In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging assay. *Nature and Science*. 2010; 8(4):22-8.

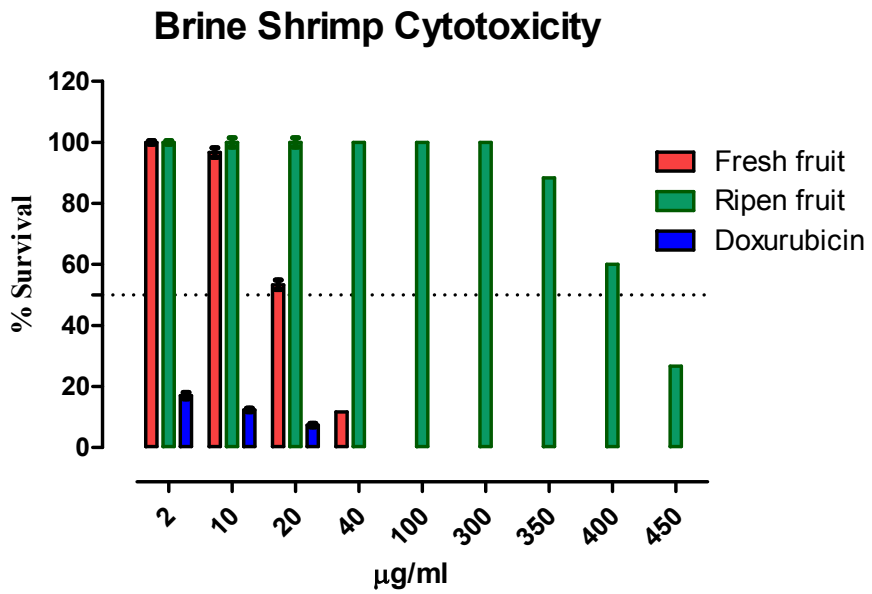

Figure 1. Cytotoxicity bioassay of aqueous extracts of green and ripens fruits of *M. azedarach* Linn

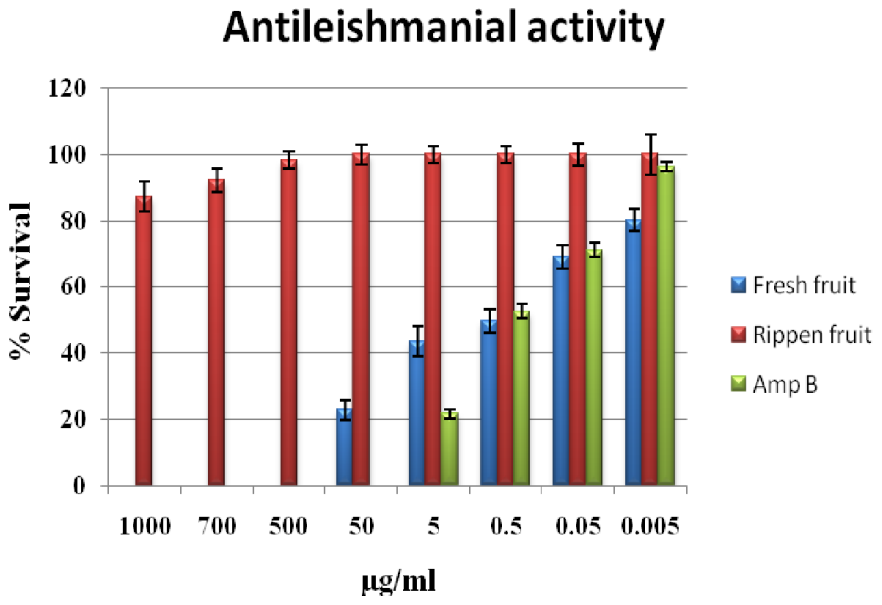

Figure 2. Antileishmanial activity of aqueous extracts of green and ripen fruits of *M. azedarach* Linn

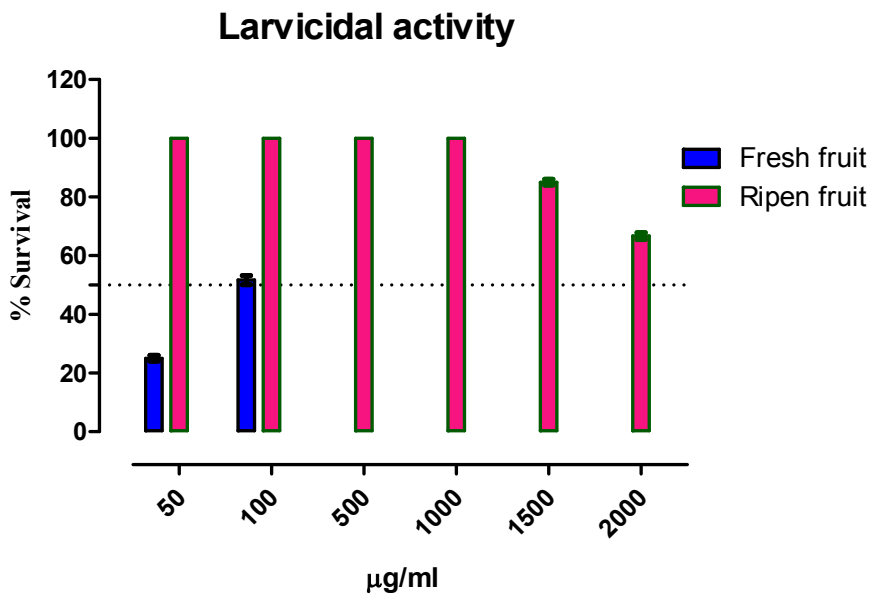

Figure 3. Larvicidal activity of aqueous extracts of green and ripens fruits of *M. azedarach* Linn. Against *Culex quinquefasciatus*

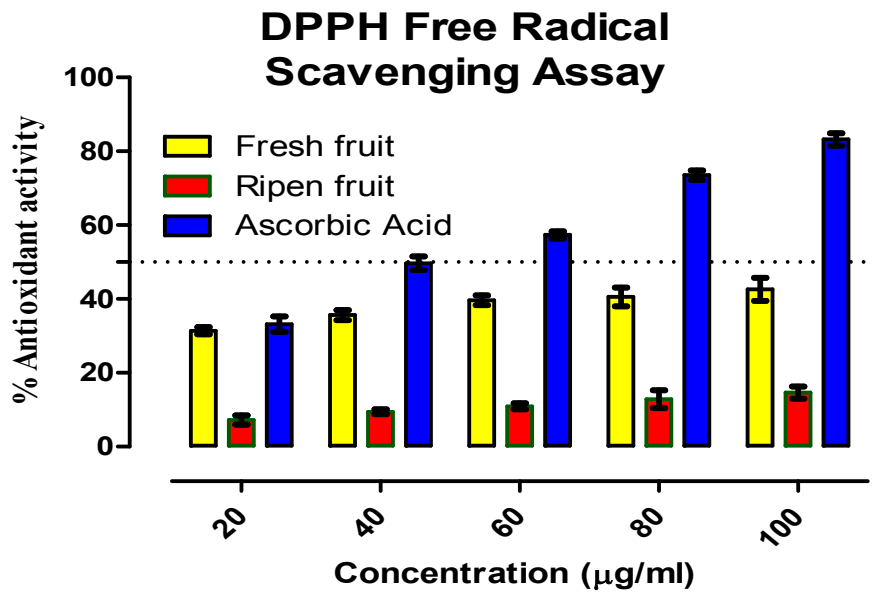

Figure 4. DPPH free radical scavenging assay of aqueous extracts of green and ripen fruits of *M. azedarach* Linn

Table 1. Antileishmanial assay of aqueous extracts of green and ripe fruits of *M. azedarach* Linn against *L. tropica*

Test sample	Antileishmanial activity of *Melia azedarach* fruits											
	% Survival											
	0.005 (µg/mL)	0.05 (ug/mL)	0.5 (ug/mL)	5 (ug/mL)	50 (ug/mL)	500 (ug/mL)	700 µg/mL	1000 (µg/mL)	LC_{50}	LC_{90}	Df	X^2
Green fruits	80.27 ± 3.21[a]	69.16 ± 3.06	49.7 ± 3.51	43.61 ± 4.50	22.7 ± 3.05	--	--	--	0.49	102.12	6	34.4
Ripen fruits	100 ± 0[b]	100 ± 0[a]	100 ± 0[a]	100 ± 0[a]	100 ± 0[a]	98.33 ± 2.64[a]	92.22 ± 3.51	87.22 ± 4.5	8227.51	>10,000	6	3.21
Amp B (Positive control)	80.38 ± 1.40[a]	71.09 ± 2.31	52.58 ± 2.14	21.57 ± 1.54	--	--	--	--	0.39	10.54	6	11.42
Distilled water/DMSO (negative control)	100[b]	100[a]	100[a]	100[a]	100[a]	100[a]	100	100	--	--	--	

*Means sharing no letter in common are significantly different at P<0.05; Means sharing same letter in common are not significantly different at P>0.05

Table 2. Brine shrimp cytotoxicity assay of aqueous extracts of green and ripen fruits of *M. azedarach* Linn

Test Samples	Concentration µg/mL	No of shrimps taken	No of shrimps killed	LC_{50} µg/mL	Lc_{90} µg/mL	X^2	Df
Green	400 350 300 100 40 20 10 02	20 20 20 20 20 20 20 20	20 ± 0 20 ± 0 20 ± 0 20 ± 0 17 ± 1.11[b] 12 ± 0.96 5 ± 1.3 0	18.07	36.52	2.7	7
Ripen	400 350 300 100 40 20 10 02	20 20 20 20 20 20 20 20	16 ± 0.43 8 ± 1.4 2 ± 1.2 03 ± 1 0 0 0 0	530.2	1480.24	22.59	7
Doxorubicin	400 350 300 100 40 20 10 02	20 20 20 20 20 20 20 20	20 ± 0 20 ± 0 20 ± 0 20 ± 0 20 ± 0 16 ± 0.98[a] 11 ± 1.5[a] 5 ± 1.3[a]	5.93	27.83	3.53	7

*Means sharing no letter in common are significantly different at $P<0.05$; Means sharing same letter in common are not significantly different at $P>0.05$

Table 3. Larvicidal activities of aqueous extract of green and ripen fruits of *M. azderach* Linn. Against Culex quinquefasciatus

Test Samples	Concentration µg/mL	No of Larvae taken	No of Larvae killed	LC_{50} µg/mL	Lc_{90} µg/mL	X^2	Df
Green	2000 1500 1000 500 100 50	20 20 20 20 20 20	20 ± 0 20 ± 0 20 ± 0 20 ± 0 10 ± 2.5[a] 6 ± 0.87[a]	67.65	142.92	13.28	4
Ripen	2000 1500 1000 500 100 50	20 20 20 20 20 20	6 ± 1.6[b] 3 ± 2.1[b] 20 ± 0 20 ± 0 20 ± 0 20 ± 0	3047.6	7171. 18	1.2	4

*Means sharing no letter in common are significantly different at P<0.05; Means sharing same letter in common are not significantly different at P>0.05

Table 4. Determination of total phenolics contents in green and ripe aqueous extract of fruits of *M. azedarach*

Varieties	Plant Parts used	Total phenolics contents (mg/g DW)
Green aqueous extract	Fruits	10.45
Ripe aqueous extract	Fruits	5.32