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## Qualitative phytochemical screening of aqueous extracts of *Azadirachta indica* and *Melia azedarach* leaves

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### Abstract

Aqueous extracts of *A. indica* and *M. azedarach* leaves were assessed for qualitative phytochemical screening by using standard laboratory methods for the presence of secondary metabolites like alkaloids, glycosides, flavonoids, reducing sugars, Terpenoids, saponins, tannins. Both the extract shown presence of alkaloids, saponins, flavonoids, tannins, phenolic compounds, glycosides, carbohydrates and terpenoids, whereas, absence of phlobatannin and proteins. The results suggested presence of phytochemicals having acaricidal properties in both the extract based on the qualitative phytochemical screening.

**Keywords:** Aqueous, alkaloids, saponins, extract, tannins

### Introduction

Plants and plant derived products have been utilised as a natural source of medicinal compounds since thousands of years. The use of natural herbal products in the treatment of animal and human diseases has gained momentum because of their safety, effectiveness, availability and inexpensiveness. In last five decades, these plants have been extensively studied by advanced scientific techniques to know the phytochemical constituents present in these medicinal plants which makes it a suitable candidate in performing numerous biological functions for curing human and animal's diseases [1].

Neem (*Azadirachta indica*) is well known Indian tree having a wide spectrum of pharmacological activities [2] and every part of neem tree have been known to possess a wide range of pharmacological properties, especially as antibacterial, antifungal, anti-malarial, anti-fertility [3], antiulcer, antifeedant, repellent, acaricidal [4], molluscicidal, ecdysone inhibitor and nematocidal [5] and is thus commercially exploitable [6, 7]. The phytochemical constituents that can be extracted from *Azadirachta indica* like alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is the main active components other than 300 isolated composites [8].

*Melia azedarach* L. is one of the most useful medicinal plants in traditional system of medicine in India. It is known for its antimicrobial, insecticidal, nematocidal, anti-inflammatory, antipyretic, antimycotic, antiulcer, spermicidal and antifertility properties [9, 10, 11, 12]. The leaves and seed of *M. azedarach* possess insecticidal, anthelmintic, antilithic, diuretic and antiseptic properties [13, 14]. Leaves and fruit extracts of *M. azedarach* were evaluated against the tick, *Rhipicephalus (Boophilus) microplus* by various workers [15, 16].

The present research paper deals with the qualitative phytochemical analysis of *Azadirachta indica* and *Melia azedarach* leaves aqueous extracts using standard laboratory methods to know their phytochemicals with acaricidal properties.

### Materials and Methods

#### Preparation of Leaves Extracts

Leaves of the plant *Azadirachta indica* and *Melia azedarach* were collected from the college campus of Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India. The collected plant materials were identified and authenticated by the expert botanist. The collected plant materials were cleaned, shade dried and then powdered using a mechanical grinder. 100 gm of powder soaked in 500 ml distilled water for 24 hours with vigorous shaking every 3-4 hours and then filtered through muslin cloth to obtain extract.

Then these extracts assessed for qualitative phytochemical analysis using standard laboratory methods.

### Qualitative Phytochemical screening

Aqueous extracts of *A. indica* and *M. azedarach* leaves were assessed for qualitative phytochemical screening by using commonly employed precipitation and coloration reaction for the presence of secondary metabolites like alkaloids, glycosides, flavonoids, reducing sugars, Terpenoids, saponins, tannins [17, 18]. Various tests such as test for alkaloids (Hager's Test and Mayer's test), test for saponins (Foam test), test for flavonoids (Shinoda Test and Sulfuric Acid Test), test for tannins (Ferric Chloride Test), test for Phlobatannins (Hydrochloric Acid Test), test for phenolic compounds (Ferric Chloride Test & Lead Acetate Test), test for glycosides (Sulfuric Acid Test), test for Carbohydrates (Benedict's Test),

test for proteins (Biuret's Test) and test for Terpenoids were carried out for the presence of the active phytochemical constituents.

### Results and Discussion

Qualitative phytochemical analysis of the crude aqueous extract of *A. indica* and *M. azedarach* leaves were carried out at Department of Pharmacology and Toxicology, PGIVAS Akola using generally accepted laboratory techniques for qualitative evaluation of Phytoconstituents [17, 18]. The screening detected the presence of bioactive principle which are believed to have acaricidal activities are saponins, tannins, phenolic compounds and alkaloids [19]. Results of qualitative phytochemical screening of crude aqueous extracts of both the plants is tabulated in the Table No. 1

**Table 1:** Phytoconstituents Present in Aqueous Extracts of *A. indica* and *M. azedarach*

Sr. No.	Phytoconstituents	Test	Plant Name	
			<i>A. indica</i>	<i>M. azedarach</i>
1.	Alkaloids	Mayer's Test	√	√
		Hager's Test	√	√
2.	Saponins	Frothing/Foam Test	√	√
3.	Flavonoids	Shinoda Test	√	√
		Sulfuric Acid Test	√	√
4.	Tannins	Ferric Chloride Test	√	√
5.	Phlobatannins	Hydrochloric Acid Test	–	–
6.	Phenolic Compounds	Ferric Chloride Test	√	√
		Lead Acetate Test	√	√
7.	Glycosides	Sulfuric Acid Test	√	√
8.	Carbohydrate	Benedicts Test	√	√
9.	Protein	Biuret Test	–	–
10.	Terpenoids	Test for Terpenoids	√	√

Note: √ Present, – Absent.



**Fig 1.1:** Alkaloids (Mayers Test)



**Fig 1.2:** Saponins (Froathing Test)



**Fig 1.3:** Flavonoids (Shinoda Test)



**Fig 1.4:** Tannins (FeCl<sub>3</sub> Test)



**Fig 1.5:** Test for Terpenoids

**Fig 1:** Qualitative Analysis of Aqueous Extracts of *A. indica* and *M. azedarach* Leaves

From the results of phytochemical screening aqueous leaves extracts of *A. indica* and *M. azedarach* found positive for the presence of alkaloids (Fig. 1.1), saponins (Fig. 1.2), flavonoids (Fig. 1.3), tannins (Fig. 1.4), phenolic compounds, glycosides, carbohydrates and terpenoids (Fig. 1.5), whereas, phlobatannin and proteins were absent in both the extracts.

These results are in accordance with results of previous workers [20, 21, 22, 23], who also found presence of saponins, flavonoids, phenols, tannins, alkaloids, glycosides, proteins, triterpenoids, carbohydrates, and alkaloids in extract of *A. indica* leaves. However, some workers found presence of these phytochemicals in higher quantity in methanolic leaf extracts of *Azadirachta indica* as compared to aqueous leaf extract [1] and complete absence of alkaloids and flavonoids in the neem leaf extract [24]. Our study also reported absence of phlobatannin and proteins in both the extracts. Alkaloid, glycoside, flavonoid and steroid whereas saponin was not present in the neem leaf sample [25]. Absence of flavonoids, saponins and phlobatannins in ethanolic leaves extract of *Azadirachta indica* were also reported [18].

Evaluated extracts of *M. azedarach* found positive for presence of flavonoids, phenolic compounds, triterpenoids, tannins and saponins [26, 27]. The reason for these variations may be due to chemical make-up of individual neem trees that may vary based on genetic and environmental factors [28]. The bioactivity of extracts varies according to concentrations of bioactive compounds, seasonality and parts of the plant from

which the compounds are extracted [29, 30]. Research findings also supported these differences may be associated with textural, chemical and nutritive soil factors that vary between geographic locations [1, 31, 32]. The screening detected the presence of bioactive principle which are believed to have acaricidal activities [20].

### Conclusions

The results of qualitative phytochemical screening of aqueous extracts of *A. indica* and *M. azedarach* leaves shown presence of saponins, tannins, phenolic compounds and alkaloids suggestive of acaricidal potential of both the plants.

### References

1. Dash SP, Dixit S, Sahoo S. Phytochemical and Biochemical Characterizations from Leaf Extracts from *Azadirachta indica*: An Important Medicinal Plant. *Biochem Anal Biochem*. 2017;6:323. doi: 10.4172/2161-1009.1000323.
2. ICAR. World Neem Conference Souvenir ICAR, Bangalore, India, 1993.
3. Subapirya R, Nagini S, Medicinal properties of Neem leaves: A review. *Curr Med Chem Anticancer Agents*. 2005;5:149-156.
4. Abdel-Shafy S, Zayed AA. *In vitro* acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum*



- excavatum* (Ixodoidea: Ixodidae). Vet Parasitol. 2002;106:89-96.
5. Sharma V, Walia S, Kumar J, Nair MG, Parmar BS. An efficient method for the purification and characterization of nematicidal azadirachtins A, B and H, using MPLC and ESIMS. J Agric Food Chem. 2003;51:3966-3972.
  6. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological Activities and Medicinal Properties of Neem (*Azadirachta indica*). Curr. Sci. 2002;82:1336-1345.
  7. Das BK, Mukherjee SC, Murjani O. Acute toxicity of neem (*Azadirachta indica*) in Indian major carps. J Aquac. Trop. 2002;17:23-33.
  8. Alves PD, Brandao MGI, Nunan EA, Vianna-Soares CD. Chromatographic evaluation and antimicrobial activity of Neem (*Azadirachta indica* A. Juss., Meliaceae) leaves hydroalcoholic extracts. Brazilian Journal of Pharmacognosy. 2009;19(2B):510-515.
  9. Schmidt GH, Rembold H, Ahmed AAI, 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- 291.
  10. Hammad AEM, Zournajian H, Talhouk S. Efficacy of extracts of *Melia azedarach* L. callus, leaves and fruits against adults of the sweet potato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). J Appl Entomol. 2001;125:483-488.
  11. Gajmer T, Singh R, Saini RK, Kalidhar SB. 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.) (Lepidoptera: Noctuidae). J Appl Entomol. 2002;126:238-243.
  12. Banchio E, Valladares G, Defago M, Palacios S, Carpinella C. Effects of *Melia azedarach* (Meliaceae) fruit extracts on the leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae): assessment in laboratory and field experiments. Ann Appl Biol. 2003;143:187-193.
  13. Khan AV. Ethnobotanical studies on plants with medicinal and anti-bacterial properties [dissertation]. Aligarh: Aligarh Muslim University, 2002, pp. 1-293.
  14. Khan AV, Khan AA, Shukla I. *In vitro* antibacterial potential of *Melia azedarach* crude leaf extracts against some human pathogenic bacterial strains. Ethnobot Leaflets. 2008;12:39-45.
  15. Dantas DA, Maganha M, Beretta TE, Nozu P, Pereira G, Da S, et al. Estudo fitoquímico dos frutos de *Melia azedarach* L. (Cinamomo, Meliaceae). In: Encontro de Pesquisa e Iniciacao Cientifica da Uniderp, Campo Grande. UNIDERP, Campo Grande, 2000, pp. 119-120.
  16. Borges LMF, Ferri PH, Silva WJ, Silva WC, Silva JG. *In vitro* efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. Medical and Veterinary Entomology. 2003;17:228-231.
  17. Kokate Kokhale, Purohit, Prachiril. Pharmacognosy. Nirali Prakashan, 2001.
  18. Demisse H, Wgebrial S. Phytochemical screening and *in-vitro* acaricidal activity of three herbal extracts against cattle tick *Boophilus decoloratus*. Int. J Pharmacognosy. 2018;5(9):596-604.
  19. Kemal J, Zerihun T, Alemu S, Sali K, Nasir M, Abraha A, et al. *In vitro* Acaricidal Activity of Selected Medicinal Plants Traditionally Used against Ticks in Eastern Ethiopia. Journal of Parasitology Research. 2020; Article ID 7834026:1-10. <https://doi.org/10.1155/2020/7834026>
  20. Timothy SY, Goji SY, Abdussalam B, Mava Y, Galadima IH. Antibacterial and phytochemical screening of the ethanolic leaf extract of *Azadirachta indica* (neem) (Meliaceae). Int. J Appl. Biol. Pharmaceut. Technol. 2011;2(3):194-199.
  21. Pandey G, Verma KK, Singh M. Evaluation of phytochemical, antibacterial and free radical scavenging properties of *Azadirachta indica* (Neem) Leaves. Int J Pharm Pharm Sci. 2014;6(2):444-447.
  22. Sharma A, Patel S. Preliminary phytochemical screening and quantitative analysis of secondary metabolites of *Mentha arvensis* and *Azadirachta indica*. International Journal of Advanced Research and Development. 2018;3(1):114-118.
  23. Khanal S. Qualitative and Quantitative Phytochemical Screening of *Azadirachta indica* Juss. Plant Parts. Int. J Appl. Sci. Biotechnol. 2021;9(2):122-127.
  24. Nwosu CO, Ugwu CE, Maduka HCC, Maduka AA, Mbaya A, Sule LI, et al. Comparative Antiparasitic Effect of Neem (*Azadirachta indica*) Leaf and Stem Bark Aqueous Extracts on Ticks (*Hyalomma* Species). Asian Journal of Ethnopharmacology and Medicinal Foods. 2016;02(05):32-37.
  25. Uwague A. Comparative Potential Qualitative and Quantitative Phytochemical Evaluation of Neem and *Moringa oleifera* Leaf Plants in Ozoro, Delta State, Nigeria. International Journal of Scientific Research in Science and Technology. 2019;6:269-274.
  26. Suresh K, Deepa P, Harisaranraj R, Achudhan V. Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. Ethnobot. Leaflets. 2008;12:1184-1191.
  27. Jeba Malar TRJ, Antonyswamy J, Ponnuswamy V, Kim YO, Al-Ghamdi AA, Elshikh MS, et al. *In-vitro* phytochemical and pharmacological bio-efficacy studies on *Azadirachta indica* A. Juss and *Melia azedarach* Linn for anticancer activity. Saudi Journal of Biological Sciences. 2020;27:682-688.
  28. Maithani A, Parcha V, Pant G, Dhulia I, Kumar D. *Azadirachta indica* (neem) leaf: A review. Journal of Pharmacy Research. 2011;4(6):1824-1827.
  29. Carpinella MC, Defago MT, Valladares G, Palacios SM. Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Melia Meliaceae) with potential use for pest management. J Agric. Food Chem. 2003;51:369-374.
  30. Hohtola A. Bioactive compounds from northern plants. Adv. Exp. Med. Biol. 2010;698:99-109.
  31. De Sousa LAD, Da Costa DP, Ferri PH, Showler AT, Borges LMF. Soil quality influences efficacy of *Melia azedarach* (Sapindales: Meliaceae), fruit extracts against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). Annals of the Entomological Society of America. 2014;107(2):484-489.
  32. Saiyam R. Evaluation of *Azadirachta indica* and *Butea frondosa* extracts against gastrointestinal nematodosis in goats. M.V. Sc. thesis (Parasitology), Nanaji Deshmukh Veterinary Science University, Jabalpur, 2018.