In-vitro phytochemical and pharmacological bio-efficacy studies on Azadirachta indica A. Juss and Melia azedarach Linn for anticancer activity

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In this study, phyto-constituents, anti-bacterial and anticancer activity of Azadirachta indica A. Juss and Melia azedarach Linn was analyzed. High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) fingerprint profile of methanol extract of A. indica and M. azedarach was carried out. The present findings showed the presence of phytochemicals such as, steroids, alkaloids, phenols, flavonoids, saponins, tannins, anthraquinone and aminoacids in A. indica and M. azedarach extracts. HPLC profiling of methanolic extract of A. indica and M. azedarach revealed eleven and ten fractions of compounds were visualized in the form of peak. In TLC methanolic extract of A. indica was separated by eight distinct phenolic and three steroidal bands and M. azedarach showed sixteen distinct phenolic and three different steroidal bands. In antibacterial activity, Among the various extracts 50 μg/ml methanolic extracts of A. indica showed high activity against K. pneumoniae (14 mm) and M. azedarach showed high activity against S. aureus (15 mm). The results suggest that the crude methanolic extracts of A. indica and M. azedarach possess significant phytochemical properties compared to other extracts and hence the phytochemicals of M. azedarach and A. indica can be exploited for plant based anticancer and antimicrobial agents in the near future. © 2019 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Medicinal plants have been widely used for the preparation of indigenous medicines and various medicinal plants are used for the formulations of Western medicines. In recent years, various medicines derived from medicinal plants are used to treat various diseases and this is not same in earlier times (Chimeray et al., 2009; Sultan et al., 2007). The continuous application of various antibiotics rise resistance among various human pathogens. Methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci and Mycobacterium tuberculosis are recently recognized as the most difficult hospital associated infections to treat and control. Medicinal plants are widely used to treat these bacterial infections against, Staphylococcus aureus, vancomycin-resistant enterococci, Mycobacterium tuberculosis and K. pneumonia (Ahmad and Beg, 2001; Agil and Ahmad, 2007; Nostro et al., 2001). Melia azadirachta Linn and Azadiracta indica A. Juss showed novel activity against various Gram-positive and Gram-negative bacteria and have been reported by Ebong et al. (2008) and Upadhyay et al. (2010). Many polyphenolic compounds were reported from the family, Meliaceae and these compounds have
potential antioxidant properties. Phytochemicals from *A. indica* are various biological properties, especially antibacterial and anticancer activity. This plant family contains various antibacterial properties especially against drug resistant bacterial pathogens (Preethi et al., 2010; Ilango et al., 2009). Many medicinal plants show anticancer properties against various cell lines. The phytochemicals from *Azadirachta indica* (Neem) have antimicrobial activity against various pathogenic organisms (Raut et al., 2014; Koon and Budida, 2011). Also, the potential anticancer property of *Azadirachta indica* was reported by Moga et al. (2018).

Cancer is the one of the important diseases and cause more death throughout the world (Rao, 2010). This disease cause serious clinical implications and pose potential economic and social impacts (Yan et al., 2009). Recently, tubulin was characterized from medicinal plants and it was found to be active against various types of cancers. This compound has various molecular mechanisms and affect microtubule depolymerisation and tubulin polymerization and hinder cell division and leads to apoptosis (Ronakzahan et al., 2011). Use of unambiguous chemicals to prevent the development or slow down the progression of carcinogenesis, the chemoprevention method, offers a promising strategy for cancer prevention (Desoize, 2004). Epidermal evidences suggest the importance of fibres in the treatment of cancer (Preethi et al., 2010). Plant nutrients and non-nutritive materials showed anticancer activity and has been proved in vivo and in vitro methods (Maiti et al., 2009). In drug discovery, medicinal plants are very much used to isolate novel bioactive compounds against cancer. More than 75% anti-infectious drugs and 60% anticancer drugs approved by FDA are derived from medicinal plants. The screened polyphenols from various medicinal plants showed anticancer properties against various cell lines (Gibellini et al., 2010). Also, flavonoids derived from medicinal plants showed anticancer properties (Mavundza et al., 2010).

*Azadirachta indica* A. Juss has potential biological properties and effective against various bacterial, fungal infections, dental disorders, skin diseases, leprosy, syphilis, malaria and also has antiseptic property (Ismail et al., 2010; Demiray et al., 2009). About 135 novel compounds with various chemical structures were determined from different parts of this medicinal plant (Hayat et al., 2010), however, very few compounds have been studied for its pharmacological potential.

Flavanoids showed anti-inflammatory and antiulcer activities and reported from medicinal plants (Ghimray et al., 2009). *Azadirachta* is a triterpenoid of the class of limonoids, found in the trees of *A. indica* (Aladakatti et al., 2010). *A. indica* has been used as insect repellent, and also used to treat various skin infections such as, ringworm, eczema, alopecia, scabies, ticks, urticaria and lice in animals (Nahak and Sahu, 2010). *A. indica* also used as antifungal, antibacterial and antiviral agents (Olabinri et al., 2009), antiatherosclerotic activity, antidiabetic activity, antimalarial activity (Omale and Okafor, 2008), antinoceptive activity, antiulcer activity, cardiovascular activity, hepatoprotective activity (Shukla et al., 2009), antitumour activity, growth regulatory activity and insecticidal activity (Galani et al., 2010; Balamurugan, 2015; Kannan and Agastian, 2015; Rathi et al., 2015).

*Melia azedarach* Linn is abundant in almost all countries and it is similar to Neem trees. Alkaloids are the predominantly present in the inner bark and it is widely used as anthelmintic. This plant also shows the properties such as, antiscorbutic, antimalarial, antifungal, antibacterial, antifeedant activity (Brandenbrug 2008; Vishnukanta and Rana, 2008). *M. azedarach* also has analgesic activity by various molecular mechanisms and has been reported by Abdelouahib et al. (2009). These leaves of this medicinal plant inhibit phagocytosis and respiratory burst and reported by Torey et al. (2010). The stem extracts of *M. azedarach* induced larval mortality and insecticidal activity (Upadhayay et al., 2010). In a study, a novel peptide Meliacine, characterized from leaves showed inhibitory effect and effectively inhibit the multiplication of foot and mouth disease virus (Salib et al., 2008).

2. Materials and methods

2.1. Collection of plant material

The aerial parts of *Azadirachta indica* A. Juss and *Melia azedarach* Linn were collected and washed with tap water and air dried. The dried plant materials were powdered and stored for analysis.

2.2. Extraction of phytochemicals

Dried plants powder (10 g) was extracted with various solvents such as, ether, petroleum, methanol, hexane and water. All extracts were kept at dark for 3 days and shaken intermittently. The extract was filtered using Whatman Number 1 filter paper and the filtrate was evaporated.

2.3. Phytochemical analysis

The extracted phytochemicals from the selected medicinal plants, *Melia azedarach* and *Azadirachta indica* were screened to determine the presence of steroids, alkaloids, phenols, flavonoids, saponins, tannins, anthraquinone and aminoacids (Wagner and Ulrich-Merzenich, 2009; Russell and Morris, 1982; Costa et al., 2010).

2.4. Isolation of bioactive compounds using High Performance of Liquid Chromatography (HPLC)

High Performance of Liquid Chromatography (HPLC) analysis was carried out using a Shimadzu LC – 10 AT VP HPLC system. Elution of bioactive compounds is performed using methanol as a mobile phase, which was filtered previously. 20 μl sample was injected manually and detected the active principles using a UV–Vis detector at 254 nm (Mallikharjuna et al., 2007; Sharanabasappa et al., 2007).

2.5. Separation of phenols and steroids using Thin Layer Chromatography (TLC)

The methanolic extracts were subjected to separation by using Thin Layer Chromatography (TLC). TLC studies for phenols and steroids were carried out by the method of Preethi et al. (2010).

2.6. In vitro anti cancer activity

Anticancer property of plant extracts were tested against MCF cell lines. It was procured from NCCS, Pune, India. It was sub cultured on to microtitre plates and used for further studies. Anti-cancer activity of medicinal plants was determined on MCF cell lines at various concentrations (50, 100, 150, 200 μg/ml). The percentage of viability and inhibition was calculated.

2.7. MITT assay

MITT assay was performed as suggested by Amer et al. (2010) with little modifications. The purified fractions were subjected for MITT assay.
2.8. Analysis of antibacterial property

Disc diffusion method was used for the screening of antibacterial activity of medicinal plants. The methanolic extract from *A. indica* and *M. azedarach* were screened for antibacterial studies against selected bacteria such as, *E. coli*, *K. pneumonia*, *P. aeruginosa* and *S. aureus* (Koona and Budida, 2011). Commercially available antibiotic disc Amphicillin was implanted along with the crude extract disc on the surface of the Muller-Hinton agar plates which is used as a positive control.

3. Results

3.1. Phytochemical components

In the present study, preliminary phytochemical screening of eight different metabolites (steroids, saponins, phenols, tannin, alkaloids, anthraquinone, amino acids and flavanoids) were tested in four different extracts. Experiments revealed the presence of steroids, saponins, tannin, anthraquinone, amino acids, flavanoids, phenols and alkaloids. *A. indica* extracts showed alkaloids, saponins, anthraquinones, flavanoids and tannins, whereas aqueous extract showed the presence of alkaloids, steroids, flavanoids, saponins. Methanolic fraction of *M. azedarach* showed steroids, phenolics, anthraquinones, flavanoids and tannins (Table 1).

3.2. Separation of compounds using HPLC

HPLC analysis was performed for the isolation of compounds from the plant samples of *A. indica* and *M. azedarach* (Figs. 1 and 2). Eleven major peaks were analyzed and the second peak was obtained at 2.107 min and it showed higher concentration (34.8%), while the sixth peak at 4.830 min showed lowest intensity (0.2%). Likewise, HPLC profile of methanol extract *Melia azedarach* was measured at 254 nm. Ten fractions of compounds were observed and the third peak showed high intensity at 2.413 min (40.3%), whereas, 8th peak showed with 11.877 min retention time showed least intensity (0.7%).

3.3. TLC profiling of steroids and phenols

3.3.1. Separation of phenols

*A. indica* extract was separated by eight distinct phenolic bands with different RF values 0.118, 0.152, 0.237, 0.254, 0.542, 0.711, 0.813 and 0.898. *Melia azedarach* separated by sixteen distinct phenolic bands were observed with different RF values 0.377, 0.754, 0.094, 0.132, 0.169, 0.226, 0.283, 0.358, 0.452, 0.566, 0.660, 0.792, 0.830, 0.849, 0.943 and 0.981. All the bands were golden yellow in colour and visualized only after iodine spray (Table 2).

3.3.2. Separation of steroids

The steroids from *A. indica* were separated by three different bands with RF values, 0.05, 0.66 and 0.466. *Melia azedarach*

![HPLC Fingerprint profiling of Azadiracta indica.](image-url)
separated by three different steroidal bands with RF values 0.036, 0.109 and 0.1818 cm. All the bands were blueish green in colour and visualized only in the presence of iodine vapour (Table 3).

### 3.4. Anticancer activity

Figs. 3 and 4 illustrated the anticancer property of methanolic extract on aerial parts of *A. indica* and *M. azedarach* that inhibited MCF cell lines at different concentration (50, 100, 150, 200 µg/ml). IC50 values of solvent extract of aerial parts of *A. indica* and *M. azedarach* were 165.5629 and 280.8989 µg/ml respectively. 200 µg/ml of the methanolic extract of *A. indica* revealed highest percentage of inhibition of 65.5% and lowest viability activity of 60.4%. In comparison to these methanolic extract of *M. azedarach* showed highest percentage of inhibition of 47.05% in 200 µg/ml of plant extract and lowest viability activity of 68%. In our study, IC50 value and percentage of inhibition of the methanolic extracts of *A. indica* showed high cytotoxic activity than *M. azedarach* (Table 4).

### 3.5. Antibacterial activity

In *A. indica*, 50 µg/ml of methanolic extract proved highly effective against *K. pneumoniae* (14 mm). However, moderate activity was observed in 50 µg/ml of the methanolic fraction of *A. indica* against *S. aureus* (9 mm) and *E. coli* (6 mm). Antibacterial activity of *M. azedarach* was screened in methanolic extract at five different concentrations against four different pathogens. Among these methanolic extract at 50 µg/ml concentration from *M. azedarach* showed high activity against *S. aureus* (16 mm). *P. aeroginosa* (12 mm) showed moderate sensitivity to methanolic extract of *M. azedarach*. *E. coli* showed lowest sensitivity to methanolic extract (5 mm) and no activity was seen in 10, 20, 30 µg/ml of methanolic extract of *M. azedarach* (Table 5).

### 4. Discussion

In the present study, two medicinal plant species *A. indica* and *M. azedarach* were used to evaluate for its antibacterial and anticancer properties. These two medicinal plants have the potential to inhibit the growth of various drug resistant bacterial species. In our study, we observed the presence of various phytochemicals and these phytochemical showed antibacterial and anticancer activities (Antonisamy et al., 2015). In a study, Timothy et al. (2011) screened the presence of various phytochemicals, including, flavonoids, sugar, terpenoids and the absence of anthroquinones in ethanolic extract of *A. indica*. In the present study methanolic

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**Table 2**

| RF Values | Melia azedarach | Azadirachta indica |
|-----------|-----------------|--------------------|
| 0.03      | +               | –                  |
| 0.07      | +               | –                  |
| 0.09      | +               | –                  |
| 0.11      | –               | +                  |
| 0.13      | +               | –                  |
| 0.16      | +               | +                  |
| 0.23      | +               | +                  |
| 0.25      | –               | +                  |
| 0.28      | +               | –                  |
| 0.35      | +               | –                  |
| 0.45      | +               | –                  |
| 0.54      | –               | +                  |
| 0.56      | +               | –                  |
| 0.66      | +               | –                  |
| 0.71      | –               | +                  |
| 0.79      | –               | +                  |
| 0.81      | –               | +                  |
| 0.84      | +               | –                  |
| 0.89      | –               | +                  |
| 0.94      | +               | –                  |
| 0.98      | –               | +                  |
| Total     | 16              | 8                  |

**Table 3**

| RF Values | Melia azedarach | Azadirachta indica |
|-----------|-----------------|--------------------|
| 0.03      | +               | –                  |
| 0.05      | –               | +                  |
| 0.07      | –               | +                  |
| 0.10      | –               | +                  |
| 0.18      | +               | –                  |
| 0.46      | –               | +                  |
| Total     | 3               | 3                  |

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Fig. 2. HPLC Fingerprint profiling of *Melia azedaracta*. 
extract of *A. indica* contains various phytochemicals including, phenolics, anthroquinones, flavanoids and tannins. Likewise, Suresh et al. (2008) reported the phytochemicals such as, flavanoids, phenolic compounds, triterpenoids and absence of catechols in the chloroform extract of *M. azedarach*. In this study, methanolic fraction *M. azedarach* revealed the presence of many phytochemicals viz., steroids, phenolics, anthroquinones, flavanoids and tannins. Hexane extract showed the presence of only two compounds (aminoacids and flavanoids). Rajapandiyan et al. (2011) revealed the antibacterial activity of *A. indica* in five different extracts (Hexane, chloroform, Ethyl acetate, alcohol and aqueous) in four different concentration (800, 1000, 1200, 1400 µg/ml). Among these, 1400 µg/ml of chloroform extract of *A. indica* showed high activity against *P. vulgaris*.

In a study, Sen and Batra (2012) reported the antibacterial property of *M. azedarach* of against various bacterial pathogens. Among these, ethanol extract of *M. azedarach* was found to be active against *P. aeruginosa* and *E. coli* and lowest activity was reported in aqueous extract of *M. azedarach* against *E. coli* (8.5 mm) and *S. aureus* (8.2 mm). In the present study, *E. coli* showed lowest sensitivity to methanolic extract (5 mm) and no activity was observed in 10, 20, 30 µg/ml of methanolic extract of *M. azedarach*. In a study, Priscila et al. (2009) observed the HPLC profiling of extracts of *A. indica* and observed two major elution peaks, at retention time, 7.933 min and 8.780 min. In our study, the methanolic fraction of the compounds was eluted between 2.413 min and 11.877 min. Ghosh et al. (2009) reported the TLC profiling of *A. indica* and noted the presence of a band at

![Fig. 3. IC 50 value of Azadiracta indica.](image)

![Fig. 4. IC 50 value of Melia azedarach.](image)
the Rf value of 0.527. In our study, methanolic extract of \textit{A. indica} showed eight distinct phenolic bands and three different steroidal bands with varied range of Rf values of 0.118–0.898 and 0.05–0.466 cm. In the present study \textit{invitro} anticancer activity was evaluated in crude methanolic extract on aerial parts of \textit{A. indica} and it inhibited MCF cell lines and had anticancer activity at different concentration. \textit{Amer et al. (2010)} assessed anticancer activity of \textit{A. indica} at different concentrations of extract. \textit{Nikoletta et al. (2010)} stated the \textit{invitro} cytotoxic property of methanolic fraction of \textit{M. azedarach} in cell line A549.

5. Conclusion

The potential anti-bacterial and anticancer activity of \textit{Azadirachta indica} \textit{A. Juss} and \textit{Melia azedarach} \textit{Linn} was analyzed. The present findings showed the presence of phytochemicals such as, steroids, alkaloids, phenols, flavonoids, saponins, tannins, antherquinone and aminoacids in \textit{A. indica} and \textit{M. azedarach} extracts. Methanolic extracts of \textit{A. indica} showed high activity against \textit{K. pneumonia}, whereas, \textit{M. azedarach} was found to be active against \textit{S. aureus}. The medicinal plants, \textit{Azadirachta indica} \textit{A. Juss} and \textit{Melia azedarach} \textit{Linn} can be effectively utilized as natural medicine to treat various bacterial infections. Also, these two medicinal plants can be effectively used as anticancer agents.

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