Saudi Journal of Biological Sciences 27 (2020) 682–688

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

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

T.R.J. Jeba Malar [a,b,*], J. Antonyswamy [a], Ponnuswamy Vijayaraghavan [c], Young Ock Kim [d], Abdullah A. Al-Ghamdi [e], Mohamed S. Elshikh [e], Ashraf A. Hatamleh [e], Monerah A. Al-Dosary [e], Sae Won Na [f], Hak-Jae Kim [g,*]

[a] *Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai 627 002, Tamil Nadu, India*
[b] *Department of Nutrition and Dietetics, Muslim Arts College, Thiruvithancode 629174, Tamil Nadu, India*
[c] *Bioprocessing Engineering Division, Smykon Biotech Pvt. LtD, Nagercoil, Kanyakumari District, Tamil Nadu, India*
[d] *Department of Bio-Environmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, 99 Daehak-Ro, Yuseung-Gu, Daejeon 34134, Republic of Korea*
[e] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[f] *The Comfort Animal Hospital, Sungbuk-gu, Soonginro-50, Seoul, Republic of Korea*
[g] *Department of Clinical Pharmacology, College of Medicine, Soonchunhyang University, Cheonan, Republic of Korea*

ARTICLE INFO

Article history:
Received 19 October 2019
Revised 5 November 2019
Accepted 19 November 2019
Available online 27 November 2019

Keywords:
Medicinal plants
Azadirachta indica
Melia azedarach
Phytochemicals
Anticancer

ABSTRACT

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. azaderach* 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. azaderach* 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.

1. Introduction

Medicinal plants have been used widely 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 (Ghimeray et al., 2009; Sultana 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; Aqil 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

* Corresponding authors at: Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai 627 002, Tamil Nadu, India (T.R.J. Jeba Malar).
E-mail addresses: renibjoy@gmail.com (T.R.J. Jeba Malar), hak3962@sch.ac.kr (H.-J. Kim).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.11.024
1319-562X/

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; Koona 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 (Maity 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 (Ghimeray et al., 2009). Azadirachtin 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), antinociceptive 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, anticancer, antimalarial, antifungal, antibacterial, antifertility and antifeedent activity (Brandenbrug 2008; Vishnukanta and Rana, 2008). *M. azedarach* also has analgesic activity by various molecular mechanisms and has been reported by Abdelouaheb et al. (2009). The leaves of this medicinal plant inhibit phagocytosis and respiratory burst and reported by Torey et al. (2010). The stem extracts of *M. azederacta* induced larval mortality and insecticidal activity (Upadhyay 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. MTT assay

MTT assay was performed as suggested by Amer et al. (2010) with little modifications. The purified fractions were subjected for MTT 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, phenolics, Anthroquinones, flavanoids and tannins, whereas aqueous extract showed the presence of alkaloids, steroids, flavanoids, saponins. Methanolic fraction of *M. azedarach* showed steroids, phenolics, anthroquinones, 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*

Table 1
Preliminary phytochemical screening of different plant extracts of *Azadirachta indica* and *Melia azedarach.*

Type of constituents	Petroleum ether		Methanol		Hexane		Aqueous	
	A. indica	*M. azedarach*	*A. indica*	*M. azedarach*	*A. indica*	*M. azedarach*	*A. indica*	*M. azedarach*
Steroids	–	–	–	+	–	+	+	–
Alkaloids	+	+	+	–	–	–	+	–
Phenols	+	–	+	+	+	–	–	+
Flavonoids	+	–	+	+	–	+	+	+
Saponins	–	+	+	–	+	–	+	+
Tannins	–	+	+	–	–	–	–	+
Anthraquinones	–	–	+	+	–	–	–	–
Aminoacids	–	–	–	+	–	+	–	–

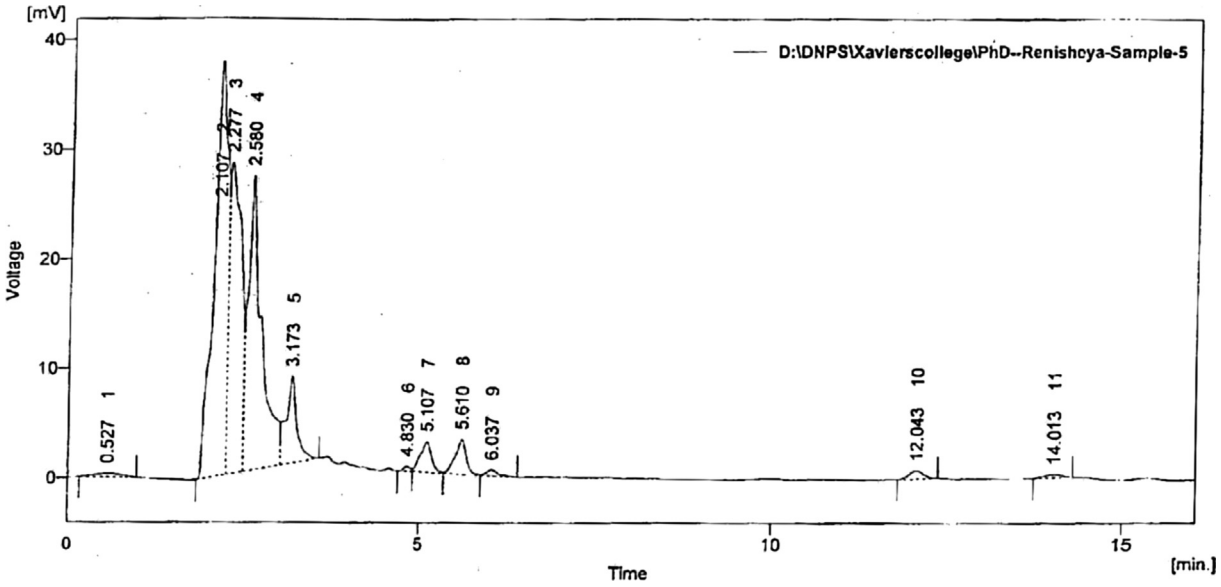

Fig. 1. HPLC Fingerprint profiling of *Azadiracta indica.*

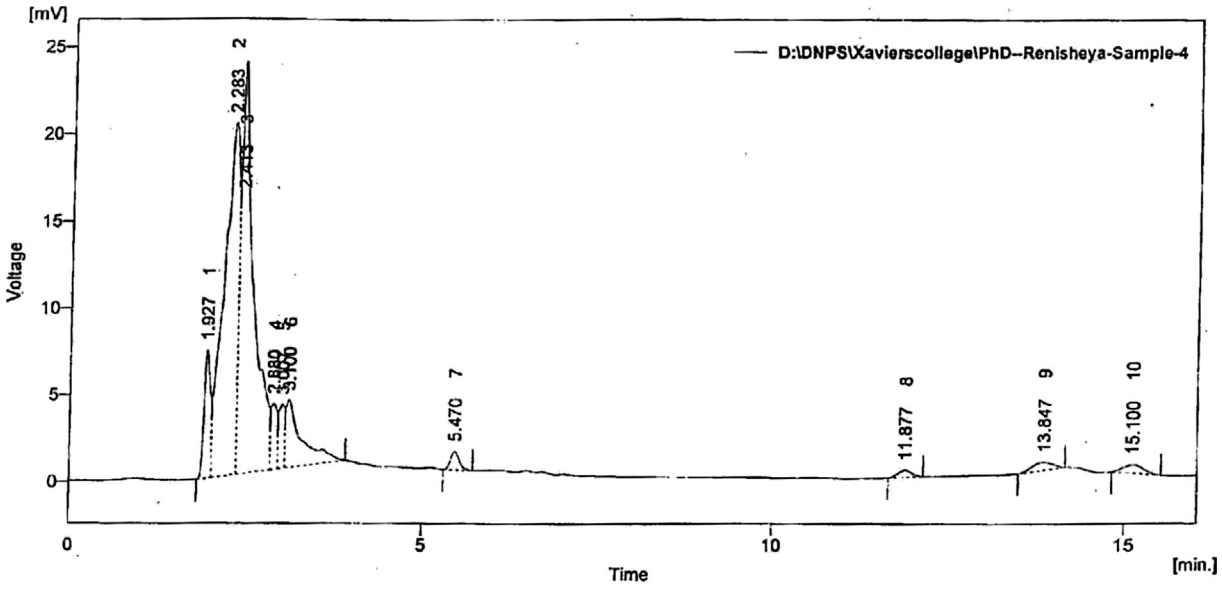

Fig. 2. HPLC Fingerprint profiling of *Melia azedaracta.*

Table 2
Separation of phenols in *Azadirachta indica* and *Melia azedarach.*

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
Separation of steroids in *Azadirachta indica* and *Melia azedarach.*

RF	*Melia azedarach*	*Azadirachta indica*
0.03	+	_
0.05	_	+
0.07	_	+
0.10	+	_
0.18	+	_
0.46	_	+
TOTAL	3	3

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. azaderach* 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. azaderach* 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. azedara*ch was screened in methanolic extract at five different concentrations against four different pathogens. Among these methanolic extract at 50 μg/ml concentration from *M. azedara*ch showed high activity against *S. aureus* (16 mm). *P. aeroginosa* (12 mm) showed moderate sensitivity to methanolic extract of *M. azedara*ch. *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. azedara*ch (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, flavanoids, sugar, terpenoids and the absence of anthroquinones in ethanolic extract of *A. indica.* In the present study methanolic

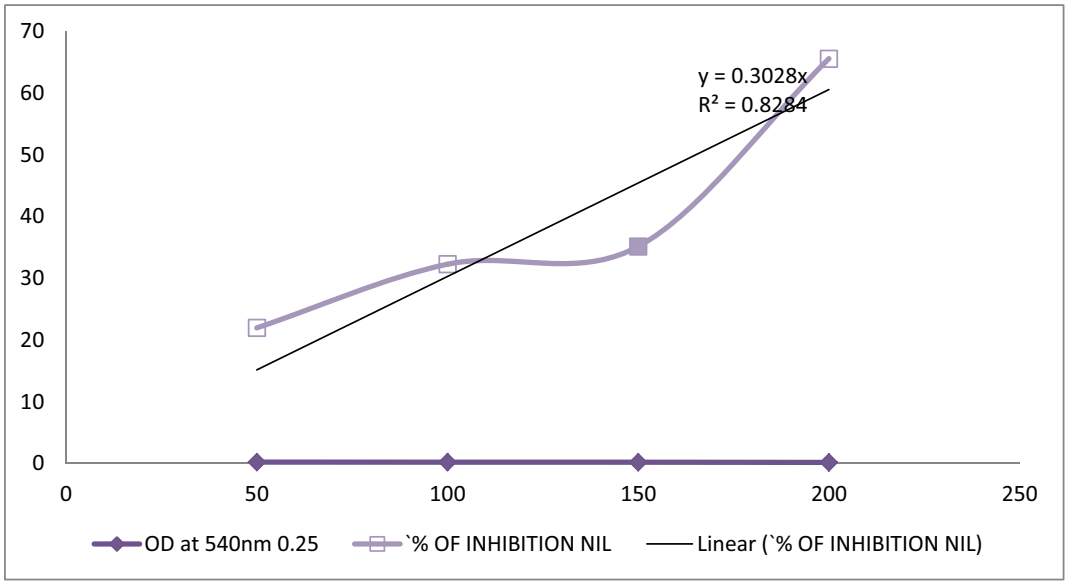

Fig. 3. IC 50 value of *Azadiracta indica.*

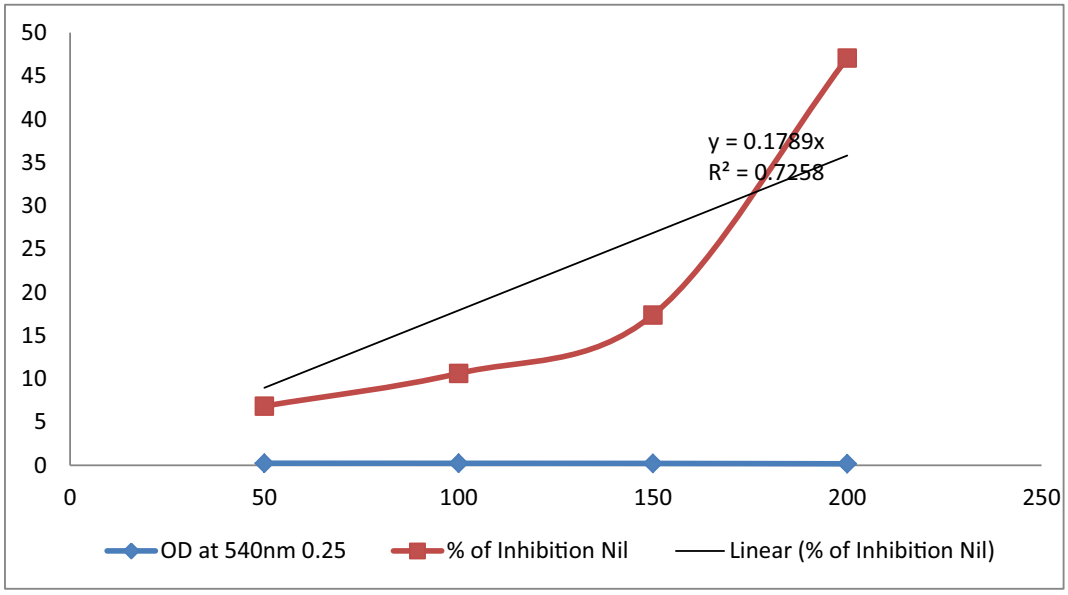

Fig. 4. IC 50 value of *Melia azedarach.*

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 faction *M. azedarach* revealed the presence of many phytochemcials 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. aeroginosa* 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. azeda*rach. 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, a sharp peak was detected from the ethanolic fractions at 2.107 and 4.830

Chiffelle et al. (2009) observed the HPLC profiling of *M. azedarach* and observed the presence of fourteen different compounds in different retention time of 2.75 to 49.23 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

Table 4
Anticancer activity of methanolic extract of *A. indica* and *M. azedarach*.

Cell line : MCF (Human Breast Cancer)									
Si no	Conc	OD at 540 nm		% viability		% of Inhibition		IC 50 VALUE	
		A. indica	*M. azedarach*	*A. indica*	*M. azedarach*	*A. indica*	*M. azedarach*	*A. indica*	*M. azedarach*
1	Control	0.250	0.250	100	100	–	–	165.5629	280.8989
2	50	0.205	0.234	82.0	93.6	21.95122	6.837607		
3	100	0.189	0.226	75.6	90.4	32.27513	10.61947		
4	150	0.185	0.213	74.0	85.2	35.13514	17.37089		
6	200	0.151	0.170	60.4	68.0	65.5629	47.05882		

Table 5
Antibacterial assay of methanolic extracts of *A. indica* and *M. azedarach*.

S.No	Test Organism	Zone of inhibition (mm)									
		10 μg/ml		20 μg/ml		30 μg/ml		40 μg/ml		50 μg/ml	
		A. indica	*M. azedarach*	*A. indica*	*M. azedarach*	*A. indica*	*M. azedarach*	*A. indica*	*M. azedarach*	*A. indica*	*M. azedarach*
1	*K. pneumonia*	–	–	5	8	8	10	12	12	14	15
2	*S. aureus*	2	2	4	6	6	9	10	14	12	16
3	*P. aeruginosa*	–	–	–	7	5	8	7	9	9	12
4	*E. coli*	–	–	–	–	2	–	4	3	6	5

the Rf value of 0.527. In our study, methanolic extract of *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 *invitro* anticancer activity was evaluated in crude methanolic extract on aerial parts of *A. indica* and it inhibited MCF cell lines and had anticancer activity at different concentration. Amer et al. (2010) assessed anticancer activity of *A. indica* at different concentrations of extract. Nikoletta et al. (2010) stated the *invitro* cytotoxic property of methanolic fraction of *M. azederach* in cell line A549.

5. Conclusion

The potential anti-bacterial and anticancer activity of *Azadirachta indica* A. Juss and *Melia azedarach* Linn was analyzed. 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. Methanolic extracts of *A. indica* showed high activity against *K. pneumonia*, whereas, *M. azedarach* was found to be active against *S. aureus*. The medicinal plants, *Azadirachta indica* A. Juss and *Melia azedarach* 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.

Acknowledgement

This work was supported by the Soonchunhyang University Research fund. The Authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No RG-1440-054. The authors also thank Department of Botany, St. Xavier's College, Smykon Biotech Pvt. LtD for the support.

References

Abdelouaheb, A., Nassima, R., Noureddine, S., 2009. Larvicidal activity of a neem tree extract (Azadiractin) against mosquito larvae in the Republic of Algeria. Jordan J. Biol. Sci. 2 (1), 15–22.
Ahmad, I., Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol. 74, 113–123.
Aladakatti, R.H., Ghodesawar, M.G., Ahmed, M., Sannadurgappa, D., 2010. Effect of lyophilized *Azadirachta indica* leaf powder on biochemical parameters of testis and epididymis in albino rats. Int. J. Biol. Chem. Sci. 6, 75–87.
Amer, H., Helmy, W.A., Taie, H.A.A., 2010. *in vitro* antitumor and antiviral activities of seeds and leaves neem *(Azadirachta indica*) extracts. Int. J. Acad. Res. 2, 477–551.
Antonisamy, P., Duraipandiyan, V., Ignacimuthu, S., Kim, J.-H., 2015. Anti-diarrhoeal activity of friedelin isolated from Azima tetracantha Lam. in Wistar rats.South Ind. J. Biol. Sci. 1, 34–37.
Aqil, F., Ahmad, I., 2007. Antibacterial properties of traditionally used Indian medicinal plants. Methods Find Exp. Clin. Pharmacol. 29, 79–92.
Balamurugan, R., 2015. Smilax chinensis Linn. (Liliaceae) root attenuates insulin resistance and ameliorate obesity in high diet induced obese rat. South Ind. J. Biol. Sci. 1, 47–51.
Brandenbrug, D., 2008. History and diagnostic significance of C-peptide. Exp. Diabetes Res., 1–7
Chiffelle, I., Huerta, A., Lizana, D., 2009. Physical and chemicals characterization of *elia azedarach* L. fruit and leaf for use as botanical insecticide. Chilean J. Agric. Res. 69, 38–45.
Costa, D.A., Chaves, M.H., Silva, W.C.S., Costa, C.L.S., 2010. Constituintes químicos, fenóis totais e atividade antioxidante de *Sterculiastriata* St Hil. et Naudin. Acta Am. 40, 207–212.
Demiray, S., Pintado, M.E., Castro, P.M.L., 2009. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tilia argentea*, *Crataegi folium* leaves and *Polygonum bistorta* roots. World Acad. Sci. Eng. Technol. 54, 312–317.
Desoize, B., 2004. Metals and metal compounds in cancer treatment. Anticancer Res. 24, 1529–1544.
Ebong, P.E., Atangwho, I.J., Eyong, E.U., Egbung, G.E., 2008. The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernoniaamygdalina* (Del.) (African bitter leaf). Am. J. Biochem. Biotechnol. 4 (3), 239–244.
Galani, V.J., Goswami, S.S., Shah, M.B., 2010. Antiulcer activity of *Trichosanthes cucumerina* linn. against experimental gastro-duodenal ulcers in rats. Oriental Pharm. Exp. Med. 10 (3), 222–228.
Ghimeray, A.K., Jin, C.W., Ghimire, B.K., Cho, D.H., 2009. Antioxidant activity quantitative estimation of azadirachtin and nimbin in *Azadirachta indica*. A. Juss grown in foothills of Nepal. African J. Biotechnol. 8 (33), 3084–3091.
Gibellini, L., Pinti, M., Nasi, M., De Biasi, S., Roat, E., Bertoncelli, L., Cossarizza, A., 2010. Interfering with ROS metabolism in cancer cells: the potential role of quercetin. Cancers 2, 1288–1311.
Hayat, K., Zhang, X., Farooq, U., Abbas, S., Xia, S., Jia, C., Zhong, F., Zhang, J., 2010. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. Food Chem. 123, 423–429.
Ilango, K., Chitra, V., Kanimozhi, P., Balaji, G., 2009. Antidiabetic, antioxidant and antibacterial activities of leaf extracts of *Adhatoda zeylanica*. Medic (Acanthaceae). J. Pharm. Sci. Res. 2, 67–73.
Ismail, H.I., Chan, K.W., Mariod, A.A., Ismail, M., 2010. Phenolic content and antioxidant activity of cantaloupe (*cucumis melo*) methanolic extracts. Food Chem. 119, 643–647.
Kannan, B.K., Agastian, P., 2015. In vitro regeneration of a rare antidiabetic plant Epaltes divaricata L. South Ind. J. Biol. Sci. 1, 52–59.
Koona, S., Budida, S., 2011. Antibacterial potential of the extracts of the leaves of *Azadirachta indica* Linn. Not. Sci. Biol. 3 (1), 65–69.
Maity, P., Hansda, D., Bandyopadhyay, U., Mishra, D.K., 2009. Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos*(L.) Corr. Indian J. Exp. Biol. 47, 849–861.

Mallikharjuna, P.B., Rajanna, L.N., Sharanabasappa, 2007. Phytochemical studies of medicinal plants. E- J. Chem. 4 (4), 510–518.
Mavundza, E.J., Tshikalange, T.E., Lall, N., Hussein, A.A., Mudau, F.N., Meyer, J.J.M., 2010. Antioxidant activity and cytotoxicity effect of flavonoids isolated from Athrixia phylicoides. J. Med. Plant Res. 4, 2584–2587.
Moga, M.A., Bălan, A., Anastasiu, C.V., Dimienescu, O.G., Neculoiu, C.D., Gavriș, C., 2018. An overview on the anticancer activity of *Azadirachta indica* (Neem) in gynecological cancers. Int. J. Mol. Sci. 19 (12), 3898.
Nahak, G., Sahu, R.K., 2010. *In vitro* antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging assay. Nat. Sci. 8 (4), 77–82.
Nikoletta, G.N., Cottiglia, F., Bueno, C.A., Alché, L.E., Leonti, M., Vargiu, S., Bifulco, E., Menkissoglu-Spiroudi, U., Caboni, P., 2010. Cytotoxic Tirucallane Triterpenoids from *Melia azedarach* Fruits. Molecules 15, 5866–5877.
Nostro, A., Bisignano, G., Cannatelli, M.A., Crisafi, G., Germanò, M.P., Alonzo, V., 2001. Effects of *Helichrysum italicum* extract on growth and enzymatic activity of *Staphylococcus aureus*. Int. J. Antimicrob. Agent. 17, 517–520.
Olabinri, B.M., Adebisi, J.A., Odesomi, O.F., Olabinri, P.F., Adeleke, G.E., 2009. Experimental classification of the antioxidant capacity of the leaf, stem and root barks of *Magnifera indica* and *Azadirachta indica*. Afr. J. Biotechnol. 8 (13), 2968–2972.
Omale, J., Okafor, P.N., 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. Afr. J. Biotechnol. 7 (17), 3129–3133.
Preethi, R., Devanathan, V.V., Loganathan, M., 2010. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. Adv. Bio. Res. 4 (2), 122–125.
Priscila, D.A., Brandão, M.G.L., Elzíria, A., Vianna-Soares, C.D., 2009. Chromatographic evaluation and antimicrobial activity of Neem (*Azadirachta indica* A. Juss., Meliaceae) leaves hydroalcoholic extracts. Rev. Bras. Farmacogn. 19 (2), 510–515.
Rajapandiyan, K., Shanthi, S., Murugan, A.M., Muthu, G.A., Singh, A.J.A.R., 2011. *Azadirachta indica* - cow urine extract, a novel controlling agent towards clinically significant multi drug resistant pathogens. J. Appl. Pharmaceut. Sci. 1 (10), 107–113.
Rao, K.N.V., 2010. Establishment of two varieties in tecoma stans of Indian origin pharmacognostically and pharmacologically. J. Phytol. 2, 92–102.
Rathi, M.A., Meenakshi, P., Gopalakrishnan, V.K., 2015. Hepatoprotective activity of ethanolic extract of *Alysicarpus vaginalis* against nitrobenzene-induced hepatic damage in rats. South Ind. J. Biol. Sci. 1, 60–65.
Raut, R.R., Sawant, A.R., Jamge, B.B., 2014. Antimicrobial activity of *Azadirachta indica* (Neem) against pathogenic microorganisms. J. Acad. Ind. Res. 3 (7), 327–329.
Ronakzahan, M., Alam, B., Saifal Islam, M., Gopal, C., 2011. Anti cancer activity of *Alangium salvifolium* flower in ehrlich ascites carcinoma bearing mice. Int. J. Cancer Res. 2011 (7), 254–262.
Russell, C.R., Morris, D.A., 1982. Invertase activity, soluble carbohydrates and inflorescence development in the tomato (*Lycopersicon esculentum* Mill). Ann. Bot. 49, 89–98.
Salib, J.Y., Michael, H.N., El-Nogoumy, S.I., 2008. New lactoyl glycoside Quercetin from Melia azedarach leaves. Chem. Nat. Comp. 44, 13–15.
Sen, A., Batra, A., 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. Int. J. Curr. Pharm. Res. 4, 67–73.
Sharanabasappa, G.K., Santhosh, M.K., Seetharam, Y.N., 2007. Phytochemical studies on *A.i ndica* and *M. azedarach* and Hard wickia binata. Roxb. E-J. Chem. 4 (1), 21–31.
Shukla, S., Mehta, A., Bajpai, V.K., Shukla, S., 2009. *In vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. Food Chem. Toxixicol. 47, 2338–2343.
Sultana, B., Anwar, F., Przybylski, R., 2007. Antioxidant activity of phenolic components present in barks *of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana* Lam.trees. Food Chem. 104 (3), 1106–1114.
Suresh, K., Deepa, P., Harisaranraj, R., Achudhan, V., 2008. 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. 12, 1184–1191.
Timothy, S.Y., Goji, S.Y., Abdussalam, B., Mava, Y., Galadima, I.H., 2011. Antibacterial and phytochemical screening of the ethanolic leaf extract of *Azadirachta indica* (neem) (meliaceae). Int. J. Appl. Biol. Pharmaceut. Technol. 2 (3), 194–199.
Torey, A., Sasidharan, S., Yeng, C., Latha, L.Y., 2010. Standardization of *Cassia spectabilis* with respect to authenticity, assay and chemical constituent analysis. Molecules 15, 3411–3420.
Upadhyay, R.K., Dwivedi, P., Ahmad, S., 2010. Antimicrobial activity of photo-activated cow urine against certain pathogenic bacterial strains. African J. Biotechnol. 9 (4), 518–522.
Vishnukanta, A.C., Rana, 2008. Melia azedarach: a phytopharmacological review. J. Pharmaco. Rev. 2, 173–179.
Wagner, H., Ulrich-Merzenich, G., 2009. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine 16, 97–110.
Yan, L.L., Zhang, Y.J., Gao, W.Y., Man, S.L., Wang, Y., 2009. *In vitro* and *in vivo* anticancer activity of steroid, saponins of *Paris polyphylla* var. *yunnanensis*. Exp. Oncol. 31 (1), 27–32.