

Application of *Azadirachta indica* (Neem) organic crude macerated extracts against postharvest decay of fruits caused by *Penicillium expansum*, *Colletotrichum gloeosporioides* and *Botrytis cinerea*

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Abstract

In present study *Azadirachta indica* (neem) leaves, seeds and twigs macerated extracts were applied as remedy to minimize fruit decay in terms of evaluating their antifungal potential against different plant pathogenic fungi: *Colletotrichum gloeosporioides*, *Penicillium expansum* and *Botrytis cinerea*. Antifungal assay against plant pathogenic fungi *B. cinerea*, *C. gloeosporioides* and *P. expansum* was carried out by well diffusion method. Percent yield, minimum inhibitory concentration, minimum fungicidal concentration, percent spore germination inhibition and percent reduction of mycelial dry weight were also determined following reported methods. The results revealed that highest zone of inhibition (22.3±0.58 mm) was shown by methanol seeds extract against *Botrytis cinerea* while n-hexane leaves and twigs extracts showed no antifungal activities. The n-hexane seed extract zone of inhibition was found to be 20±1.5 mm against *C. gloeosporioides* however n-hexane leaves and twigs extracts were inactive. Methanol seed extract have shown significant inhibition zone (23±01 mm) against *P. expansum* however chloroform twigs and n-hexane leaves and twigs extract were inactive. Leaves and seeds extract of *A. indica* inhibited the growth of spores and mycelium of tested fungal strains. About 100% SGI (total inhibition) was recorded for methanolic leaves and seeds extracts against *Botrytis cinerea* and *Sclerotinia sclerotiorum*, and n-hexane seeds extract against *Colletotrichum capsici*. The lowest SGI was recorded (36%) for twigs ethyl acetate extract.

Keywords: *A. indica*; antifungal potential; extraction yield; functional groups; phytochemicals

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Introduction

All over the world plant fungal diseases are recognized to cause huge impairments in plants and even animals. Fungi causes spoilage of seeds, grains, crops and perhaps have second position to pests as a source of postharvest losses globally. The *A. indica* has been utilized as antimicrobial agent by human beings from centuries. It is an important curative agent for allegedly over hundred ailments, an agrochemical pesticide, food, heating source, soil amendment and wood (Dauda *et al.*, 2015). Agriculture yield losses up to 90% are because of the pathogenic fungi, the main causes of the crop diseases (Sadiqullah *et al.*, 2018). Global postharvest losses of fresh vegetables are estimated to be 20-40%, limiting fruit and vegetable supply in the market. Mechanical damage during and after harvest speed up the spoilage of fruits and vegetables by fungi such as *Aspergillus*, *Alternaria*, *Cladosporium*, *Botrytis*, *Fusarium*, *Pestalotiopsis*, *Ceratocystis*, *Colletotrichum*, *Monilinia*, *Phomopsis*, *Geotrichum*, *Penicillium*, *Lasiodiplodia*, *Mucor*, *Phoma*, *Rhizopus* and *Phytophthora*. Postharvest losses are one of the primary causes of worldwide economic losses in the agricultural sector (de Oliveira Filho *et al.*, 2021). Synthetic fungicides are applied to avoid the spoilage caused by pathogenic fungi, but these fungicides have created some problems i.e. environmental pollution, development of fungal resistance and poisonous effects to fishes, animals, birds and human beings (Saba *et al.*, 2010). Therefore, it is need of the day to search for a substitute from natural origin which are ecofriendly, cheap and easily available to all farmers.

The plant under study, *A. indica*, commonly recognized as Neem; is a member of the Meliaceae family and is widely distributed in Pakistan, Bangladesh, India and Nepal. This plant has medicinal properties and is used to treat various diseases by virtue of the presence of a variety of phytochemicals such limonoids, nimbin, nimbidin, and nembolide. The first polyphenolic flavonoids with antifungal and antibacterial qualities extracted from *A. indica* leaves were quercetin and β -sitosterol (Govindachari *et al.*, 1998). To preserve dental health, *A. indica* is a traditional medicinal plant used in India (Imam *et al.*, 2012). *A. indica* has been used in traditional medicine since ancient times to treat a range of human diseases (Joshi *et al.*, 2010). Neem leaves, roots and seeds contain antibacterial and antifungal agents as reported in literature (Alzohairy 2016; Ospina *et al.*, 2015). The neem plant contained many bioactive compounds that are structurally and chemically different from each other with broad spectrum of biological potentials including antimicrobial potentials as well. According to a reported study, more than 140 compounds have been found in different parts of neem plant (Mahmoud *et al.*, 2011). Several types of phytochemicals including carotenoids, ketones, flavonoids, steroids, and phenolic compounds have been extracted from this plant (El-Hawary *et al.*, 2013).

Synthetic fungicides possess a wide range of side effects therefore, development of new fungicides of plant origin would be a best alternative which might have minimal adverse effects (Srivastava and Kumar, 2000). In the search of organic, ecofriendly and plant-based pesticides; *A. indica* (neem) is the most favorable candidate if used as fungicide. Numerous plant pathogens have been reported to be controlled by certain plant extracts including neem due to their pesticidal properties present (Benisheikh *et al.*, 2019). A number of literature reports have been published on crude extracts of neem showing fungicidal potentials against various pathogenic fungi. However postharvest decay of fruits due to fungal strains like *Penicillium expansum*, *Colletotrichum gloeosporioides* and *Botrytis cinerea* have not been documented yet. Therefore, the current research work was designed to compare and evaluate the macerated extracts as fungicidal agent in postharvest. Extraction and *in-vitro* fungicidal potential of seeds, twigs and leaves extracts of *A. Indica*, against postharvest pathogenic fungi such as *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Penicillium expansum* have been documented in this study. The major bioactive compounds categories were also documented using phytochemical tests.

Materials and Methods

Collection and processing of A. indica parts

The *A. indica* parts seeds, twigs and leaves were obtained from Khyber Bazar, Peshawar City Khyber Pakhtunkhwa-Pakistan. The collected *A. indica* or neem parts were shifted to Food Technology Center Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar Pakistan. The parts were cautiously rinsed with flowing tap water and sterilized by 1% NaOCl, then carefully washed again with distilled water. The plant material was then dried in an Air Cabinet Dryer (England) at 40 °C and pulverized to make powder using Wiley Mill Standard Model No.3-USA. The resulted powder material was placed in closed brown bottles till maceration in solvents.

Maceration extraction process and extractive % yield values

The powdered sample (10 g) was extracted with a 100 mL methanol solvent by using maceration technique for three days. Additional fractions of the solvent were poured into marc and the extraction was recurring until the final extract showed no more color. Whatman® 40 filter paper 125 mm Ø Whatman International Ltd. Maidstone- England was used to filter the sample. A rotary evaporator (B-490 Heating Bath-Buchi, R-200 Buchi Rotavapor-Switzerland) was used at 45 °C for 08 h under reduced pressure to evaporate the methanol solvent. After rotary evaporation the crude extract became a semi solid mass and shifted from evaporator flask into a beaker and kept it on hot plate at 45 °C in a fuming hood until all the solvent evaporated. Using different solvents, the extraction process was also carried out (Samuel *et al.*, 2021).

Antifungal assay

Test fungi

The fungal pathogens, *Penicillium expansum*, *Colletotrichum gloeosporioides* and *Botrytis cinerea* were obtained from the Mycotoxin Research Section of PCSIR Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan. These fungi were sub-cultured on Potato Dextrose Agar (PDA) in slants and working cultures were made in the Potato Dextrose Broth (PDB). All the cultures were stored in the cooled incubator (Gallenkamp-UK) at 10 °C for the forthcoming experimental works.

Agar well diffusion method

The sterilized PDA media were decanted in the pre-sterilized Petri dishes and solidified. Then 0.1 mL fungal spore suspensions (10^5 spores/mL) were spread uniformly with the help of sterilized glass spreader. Using sterilized cork borer wells of 6 mm diameter were punched in the PDA media. Each well was filled with each test extract portion of 100 µL from 500 mg/mL. Distilled water was used as control while Mancozeb as standard in this study. The inoculated plates were kept for 3-5 days at 25 °C in the incubator (Memmert-Germany). After incubation process the inhibition zones (mm) around the wells were calculated using Vernier Caliper (Ali and Abbas, 2023).

Minimum inhibition concentration and minimum fungicidal concentration of crude extracts against fungal strains

The minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) of *A. indica* crude extracts were screened as per protocol previous described method reported in literature (Anthony, 2000).

Evaluation of percent spore germination inhibition

The assay reduction of percent spore germination was determined using the previous reported standard procedure (Anokwuru *et al.*, 2011).

Effect of extracts on the mycelial dry weight

About 5 mL (500 mg/mL) of the respective extracts was added to 25 mL of malt extracts broth in each of 250 mL conical flasks. Control flasks were also prepared comprising of equal amounts of sterile distilled water. By means of the Cork borer holes were made and each flask was inoculated with 4mm mycelia disc from 7-day old cultures of fungi. Each flask was then incubated at 26 ± 1 °C for 7 days. Each of the fungi had five replicates per treatment. The basal medium without the extract was inoculated by same procedure. The flask with distilled water served as the control. After the period of observation, the mycelia were filtered over a pre dried and weighed filter paper in a Millipore filter connected to suction pump. The filter papers with contents were dried to a constant weight at 85 °C at hot air oven overnight. The dry weights of the mycelia were then obtained from the difference of the two weights. The experiment was carried out in five replicates but before inoculation the pH of the medium was adjusted to 6.5. The dry weights of the mycelia were calculated using the given formula described in equation 1 (Wafaa *et al.*, 2007):

$$W_m = W_3 - W_2 \quad (1)$$

Where; W_1 = dry filter paper weight

W_2 = Agar + Weight of filter paper

W_3 = Weight of dry mycelium + filter paper + agar.

Fourier Transforms Infrared (FTIR) spectrophotometric analysis

The neem twigs, seeds and leaves crude extracts different functional groups of phytochemicals were screened using the FTIR spectrophotometer (Prestige-21, Shimadzu-Japan). The Infrared (IR) range for the analysis was 500-4000 1/cm and IR peaks were observed in transmitted form.

Statistical analysis

The experimental parameters were run in triplicate ($n=3$), determining mean, average, standard deviation (SD) and standard error of the mean (SEM) using Statistical Package for the Social Sciences (SPSS) software version 21.0 NY-USA.

Results and Discussion

Extraction yield % and extraction solvents system

A diverse range of phytochemicals or compounds are present in plants, which make them a primary source of foods and medicine. Maceration extractive yield values (%) of *A. indica* are shown in Table 1. The soaking yield is a compute of the solvent effectiveness to obtain targeted molecules from the experimentally processed plant. Under numerous conditions it gives an idea about the extractability of the studied plants. Our results showed that maximum yield was obtained 22%, 14% and 13% when neem seeds were extracted by maceration technique with methanol, ethanol and ethyl acetate respectively. Similarly, leaves showed a low extraction values as compared to seeds, but high extractive value as compare with twigs. The current findings revealed that as compared to other solvents, methanol showed efficient bioactive compounds upon extraction. Each of the crude extracts gave different percent yield according to the plant parts.

Table 1. Maceration extractive yield values (%) of *A. indica*

Solvents	Seed	Leaves	Twigs
Methanol	22±02	11±0.6	05±1.5
Chloroform	04±01	02±00	02.00±00
Hexane	06±01	01±00	00.60±00
Ethanol	14.00±01	10±0.4	04±00
Ethyl acetate	13±0.7	01±00	03±00

Values expressed are mean (n=3) ± Standard deviation

In the literature findings using various solvents different percent extractive yields of neem parts has been recorded. The extraction percent yield of neem leaves and seed were found to be 17.05 and 23.92 (Samuel *et al.*, 2021). The neem stem bark extracts yield % for acetone (11.7%), ethanol (6.8%), ethyl acetate (3.43%) and methanol was 9.77% [15]. The yield extraction of seed kernel, seeds and leaves were 20.0%, 34.7% and 9.1% (n-hexane), 10.7%, 11.3% and 6.8% (chloroform) and 65.7%, 52.9% and 65.2% (methanol) (Wafaa *et al.*, 2007). A neem leaf possesses the highest oil yield 37.73% by Soxhlet technique using ethanol, while seed and bark were found to be 34.85% and 27.70% respectively (Evuomwan *et al.*, 2015). Pure ethanol produced the maximum extractive yield in neem bark as compared to pure methanol, water ethanol and methanol (Sultana *et al.*, 2009). The extractive yield of oil obtained from seeds by Soxhlet and hexane as solvent was 37% (Jessinta *et al.*, 2013). Seed oil yielded 28.4% extractive value using Soxhlet system with hexane solvent (El-Mahmood *et al.*, 2010). The yield extraction percentage variation might be accredited by the drying season, plants parts maturity, harvesting period, climate, localities, extraction methods, and solvents uses (Jessinta *et al.*, 2013).

Methanol is a best choice for the extraction of polar and non-polar substances. This produce the bioactive compounds of polar and non-polar might be pulling out easily (Norazlina *et al.*, 2021). Using ethanol as solvent in Soxhlet method the maximum yield of neem leaves extract was obtained after three hours of extraction (Tesfaye and Tefera 2021). Ethanol is willingly mix with H₂O and break down water-soluble substances such as chlorophyll due to its polar nature, other factors like temperature used during extraction and particle size of neem leave powder should be considered to obtain a better result during the extraction process (Norazlina *et al.*, 2021).

The toxicity and poisonous nature of ethyl acetate is least a similar to ethanol solvent. It was recognized that following extraction varied solvents lastly formed unlike phyto-compounds or phytochemicals (Norazlina *et al.*, 2021). This is due to the polarity of the solvent has different extraction abilities to each secondary metabolite. The minimum extractions of phenolic substances were obtained in water extracts and maximum extractions were reported in ethyl acetate (Norazlina *et al.*, 2021). Hexane is applied as solvent for extraction due to its properties such as easy recovery, high capability in selectivity to solvent, small latent heat of vaporization and non-polar nature (Norazlina *et al.*, 2021).

Qualitative assessment of phytochemicals

Phytochemicals analysis of *A. indica* maceration Leaves, seeds and twigs are presented in Table 2. From the results, it is clear that tannins, alkaloids, flavonoids and phenols are present in high concentration (+++) in methanol leaves extracts. Similarly, alkaloids, glycosides, flavonoids and phenols are present in high concentration (+++) in ethanol leaves extracts. Generally, the phytochemicals in all the extracts are absent (-) or in low concentration (+). In leaves high amount of phyto-compounds, were found followed by seeds and twigs, while the solvent extracts have observed an efficiency in the order of; methanol>ethanol>chloroform>ethyl acetate >hexane.

The qualitative analysis shows the different phytochemicals at various detected levels.

Table 2. Phytochemicals screening of *A. indica*

Bioactive compounds	Parts	Methanol	Chloroform	<i>n</i> -Hexane	Ethanol	Ethyl acetate
Saponins	Leaves	++	+	+	+	+
	Seeds	++	+	+	++	+
	Twigs	+	-	-	+	-
Tannins	Leaves	+++	++	+	++	+
	Seeds	++	+	+	++	+
	Twigs	+	-	-	+	-
Alkaloids	Leaves	+++	++	+	+++	+
	Seeds	++	+	-	+	+
	Twigs	+	-	-	+	+
Glycosides	Leaves	++	++	+	+++	+
	Seeds	++	+	+	++	+
	Twigs	+	-	-	+	-
Flavonoids	Leaves	+++	++	+	+++	+
	Seeds	+++	+	+	++	+
	Twigs	+	+	-	+	+
Phenols	Leaves	+++	++	+	+++	+
	Seeds	+++	+	+	++	+
	Twigs	+	+	-	+	+
Terpenoids	Leaves	++	++	++	++	-
	Seeds	++	+	+	++	+
	Twigs	-	-	-	-	-

Key: +++= High concentration, ++= moderate concentration, +=low concentration, - = Absent

The variation in the fungicidal activities might be because of variation allocation of different botanical molecules in various extracts and parts. Phytochemical components of neem leaf aqueous and methanol extracts have been reported by the researchers (Dash *et al.*, 2017; Bharat *et al.*, 2015) are in close agreement to our current study. While the phytochemicals in neem leaves extracts reported by (Benisheikh *et al.*, 2019; Effiong *et al.*, 2016) and neem twigs aqueous extract (Awache *et al.*, 2017) reported dissimilar results as compared to our study. This tendency may be due to disparate geological position or habitats of the herbs. The absence and presence of certain metabolites types in the neem leaves could be different because of environment characteristics and ecological location (Shuaibu *et al.*, 2015; Ghimeray *et al.*, 2009)

Antifungal activities

Antifungal activities of *A. indica* leaves, seeds and twigs maceration extracts are shown below in Table 3. Agar well diffusion technique was applied to evaluate the antifungal activities by calculating the zone diameter of inhibition in millimeter. The results of this study revealed that the methanol and ethanol extracts of seeds and leaves possess strong zone of inhibition against the tested fungal pathogenic strains. The extracts antifungal activity displayed different selectivity for each fungus studied. The values were expressed as mean±SD.

Table 3. Antifungal activities of *A. indica* leaves, seed and twigs extracts in term of zone of inhibition (mm) using different extracting solvents

Solvent	Neem parts	<i>Botrytis cinerea</i>	<i>Colletotrichum gloeosporioides</i>	<i>Penicillium expansum</i>
Methanol	Leaves	22±1.5	15±01	20.3±0.58
	Seeds	22.3±0.58	18±1.5	23±01
	Twigs	12±01	17±00	13.7±1.15
Chloroform	Leaves	19±1.5	14±02	18.3±0.58
	Seeds	14.3±2.5	15±01	17±02
	Twigs	11±0.4	13.3±1.5	-
Hexane	Leaves	-	-	-
	Seeds	18±0.8	20±1.5	19.6±0.57
	Twigs	-	-	-
Ethanol	Leaves	21±02	14.6±1.1	19.3±1.15
	Seeds	20±01	17.6±2.5	14.67±2.08
	Twigs	16.3±1.1	14.3±1.2	12±01
Ethyl acetate	Leaves	11±0.57	13±00	10±00
	Seeds	13±00	16.7±1.5	15±00
	Twigs	10±1.73	-	09±00
Negative Control		-	-	-
Positive Control		25±01	24±0.8	26±1.5

Values expressed are mean (n=3) ± Standard deviation; - = No activity

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of neem extracts against plant pathogenic fungi is shown in Table 4. The MICs and MFCs range were different according to the plant extracts parts, type of solvent and fungal strains. The lowest MICs were observed 31.25 mg/mL for methanolic leaves and seeds extracts against *Botrytis cinerea* and *Sclerotinia sclerotiorum*. These results predicted that methanol and ethanol leaves and seeds extracts have lower MICs and MFCs as compared to the other extracts. The highest MICs and MFCs values were recorded in case of ethyl acetate as extractive solvent.

Table 4. *A. indica* extracts MIC (mg/mL) and MFC (mg/mL) against plant pathogenic fungi

Solvent	Neem parts	<i>Botrytis cinerea</i>		<i>Colletotrichum gloeosporioides</i>		<i>Penicillium expansum</i>	
		MIC	MFC	MIC	MFC	MIC	MFC
Methanol	Leaves	31.25	62.5	125	250	62.5	125
	Seeds	31.25	62.5	62.5	125	31.25	62.5
	Twigs	500	1000	125	250	1000	1000
Chloroform	Leaves	125	250	250	500	250	500
	Seeds	250	500	250	250	500	500
	Twigs	500	1000	1000	1000	NA	NA
Hexane	Leaves	NA	NA	NA	NA	NA	NA
	Seeds	125	250	62.5	125	250	500
	Twigs	NA	NA	NA	NA	NA	NA
Ethanol	Leaves	62.5	125	250	500	62.5	125
	Seeds	125	250	125	250	1000	1000
	Twigs	250	500	500	1000	1000	1000
Ethyl acetate	Leaves	500	1000	1000	1000	1000	1000
	Seeds	500	1000	1000	1000	1000	1000
	Twigs	1000	1000	NA	NA	1000	1000

NA=Not Applied

Table 5 shows the effect of neem extracts (500 mg/mL) on spore germination inhibition % (SGI%) and spore germination % (SG%) against the given pathogenic fungi. Among these extracts totally inhibition (100% SGI) were recorded in methanol leaves and seeds against *Botrytis cinerea* and *Sclerotinia sclerotiorum*, 100% SGI (total inhibition) in hexane seeds extracts against *Colletotrichum capsici*. The lowest SGI were seen in the twig's ethyl acetate extracts.

Table 5. Spore germination inhibition % (SGI %) and spore germination % (SG%) of fungi exposed to neem extracts

Solvent	Neem parts	<i>Botrytis cinerea</i>		<i>Colletotrichum gloeosporioides</i>		<i>Penicillium expansum</i>	
		%SGI	%SG	%SGI	%SG	%SGI	%SG
Methanol	Leaves	100±2	00	67±1.2	33±0	100±0	00
	Seeds	100±1.8	00	85±2	15±0	100±0	00
	Twigs	58±10	42±0	75±1.5	25±01	64±1.3	36±0
Chloroform	Leaves	88±02	12±0	56±01	44±0	94±1	6±0
	Seeds	47±1.7	53±01	62±01	38±01	71±0.5	29±0
	Twigs	68±2.3	32±0	53±01	47±01	00	100±01
Hexane	Leaves	00	100±02	00	100±01	00	100±3.4
	Seeds	84±1.5	16±0	93±02	7±0	86±01	14±0
	Twigs	00	100±01	00	100±02	00	100±1.5
Ethanol	Leaves	94±1.4	6±0	54±01	46±0	82±01	18±0
	Seeds	95±2.5	5±0	72±0	28±0	65±1.3	35±0
	Twigs	65±01	35±0	62±01	38±0	56±1.5	44±0
Ethyl acetate	Leaves	55±02	45±01	58±01	42±01	55±0.9	450.7
	Seeds	60±03	40±01	68±0	32±01	64±1.7	36±0
	Twigs	45±01	55±02	00	100±0	40±0.5	60±10
Negative control	-	00	100±1.5	00	100±02	00	100±02
Positive control	-	100±0	00	100±0	00	100±0	00

Values expressed are mean (n=3) ± Standard deviation. Positive Control = Diflucan (Synthetic fungicide), Negative Control = Distilled H₂O

Effect of neem extracts concentration on dry weight of post-harvest fungi is shown in Table 6. These findings confirmed that methanol seeds extract of neem observed maximum mycelial dry weight reduction as compared to the other extracts. While *Botrytis cinerea* showed high susceptibility as compared to the other two study pathogenic fungi.

Table 6. Effect of neem extracts on dry weight of post-harvest fungi

Solvent	Neem parts	Mycelial Dry Weight (mg)		
		<i>Botrytis cinerea</i>	<i>Colletotrichum gloeosporioides</i>	<i>Penicillium expansum</i>
Methanol	Leaves	295±01	504±02	298±00
	Seeds	292±02	310±01	302±1.5
	Twigs	460±01	515±1.5	510±00
Chloroform	Leaves	380±02	515±1.5	304±01
	Seeds	510±0.5	530±01	465±00
	Twigs	507±03	533±01	547±01
Hexane	Leaves	545±01	547±02	548±1.5
	Seeds	311±01	315±00	380±00
	Twigs	550±1.5	555±03	558±1.5
Ethanol	Leaves	330±01	530±1.5	388±00

	Seeds	325±01	462±01	509±1.5
	Twigs	506±02	513±01	523±00
Ethyl acetate	Leaves	520±02	525±1.5	535±01
	Seeds	505±1.5	508±01	512±1.5
	Twigs	540±01	580±1.5	550±01
Negative control	-	580±01	600±01	590±01
Positive control	-	201±1.5	220±01	210±01

Values expressed are mean (n=3) ± Standard deviation

The antifungal effects of the plants are because of numerous kinds of phytochemicals like flavonoid, alkaloids, saponins etc. (Sheema and Durai 2014). The steroidal saponins give protection to plants against fungi and other several pathogens (Sprag *et al.*, 2004; Yang *et al.*, 2006; Saniewska *et al.*, 2005; Elisa *et al.*, 2007). In the current study, saponins may be the active phytoconstituents responsible for the fungicidal activity. Saponins display effective fungistatic capacity and are frequently occur in comparatively elevated quantity in vigorous herbs and as an outcome have been associated as sign of herb's fight against fungi (Osbourne, 1996). It can be concluded from the current findings that these secondary metabolites present in different parts of neem might be responsible for the observed fungicidal capabilities. The antifungal activity of neem leaves extracts (Benisheikh *et al.*, 2019; Abid *et al.*, 2016; Bhatnagar and Zeringue 1993; Bhatnagar and McCormick 1988); neem leaf and stem bark extracts (Effiong *et al.*, 2016); neem leaves, seed, flower, stem and fruit extracts (hexane, methanol and methanol-water 70/30 v/v) have been reported for antifungal activity against *Fusarium mangiferae* (Usha *et al.*, 2009) with dissimilar results as compared to the present study.

Our investigations point out that the phytochemicals which are either non-polar or polar, could be efficiently isolated through organic solvent system. Several previous findings showed the maximum fungicidal potential of ethanol/methanol solvents. The water extracts displayed minimum fungicidal activities, which could be accounted to quantity of the bioactive composition in the extracts and extracting capabilities of the solvent. Further many bioactive compounds are better dissolved in alcoholic solvents as compared to aqueous. Some scientists have accounted that water extracts do not display good potential against fungal strains (Narasimharaju *et al.*, 2015).

The neem antifungal activity is because of the numerous kinds of phenolics and tetra terpenoids (Khajista, 2013). The less efficiency of water as compared to ethanol extract of the neem leaves might be due to the variations in the extract's constituents (Mondali *et al.*, 2009). Neem fruit extract was found less significant than leaves extract and methanol extract of leaves possessed maximum antifungal potential (Khajista, 2013). *A. indica* active compositions comprised mainly of triterpenoides i.e. azadirachtin, nimbidine, nimbin etc. (Mondali *et al.*, 2009). The differences in antifungal potential of numerous extracts could be credited to the phytochemicals existence that are extracted by dissimilar solvents, which could be inclined by different conditions like type of extracting solvent, extraction techniques and age of plants (Ali and Abbas 2023; Mondali *et al.*, 2009; Qasem and Abu-Blam 1996). The phytochemicals saponins, flavonoids, glycosides and alkaloids are the antimicrobials doctrine of herbs. These antimicrobial principles are in fact the self-protective system of the plants against diverse pathogens and they can either constitutive components of cells or be de novo formed during the pathogenesis (VanEtten *et al.*, 1994; El-Hawary *et al.*, 2013). The mechanism behind the antimicrobial nature of the phyto-compounds is clarify by the reality that this action could be a outcomes as a result of enzymatic processes destruction concerned in energy creation (El-Hawary *et al.*, 2013) or secondary metabolites might cause structural injure of the cell membrane causing death of the cell (El-Hawary *et al.*, 2013). The secondary metabolites act on the fungi hyphal cell wall, which lead to reduce the stringency and fall down mycelium of the fungi (Sharma and Tripathi 2006). The phytochemicals cross the cell

membrane quietly into the inner of the cell disturbing vital intercellular functions like synthesis of nucleic acids (El-Hawary *et al.*, 2013).

Fourier Transform Infrared (FTIR) spectroscopic assay

The functional groups of the bioactive molecules were identified using the FTIR spectrum, which are bases of the spectrum peaks found in the vicinity of infrared radiations. FTIR spectra of methanol neem leaves extract is shown in Figure 1. Sixteen (16) spectra are appearing in this figure. 3342.64 cm⁻¹, 2920.23 cm⁻¹, 1606.70 cm⁻¹, 1361.74 cm⁻¹, 1242.16 cm⁻¹, 1199.72 cm⁻¹, 1155.30 cm⁻¹, 1049.28 cm⁻¹, 1033.85 cm⁻¹, 817.82 cm⁻¹, 691.37 cm⁻¹, 646.15 cm⁻¹, 605.65 cm⁻¹, 591.00 cm⁻¹ and 580.57 cm⁻¹.

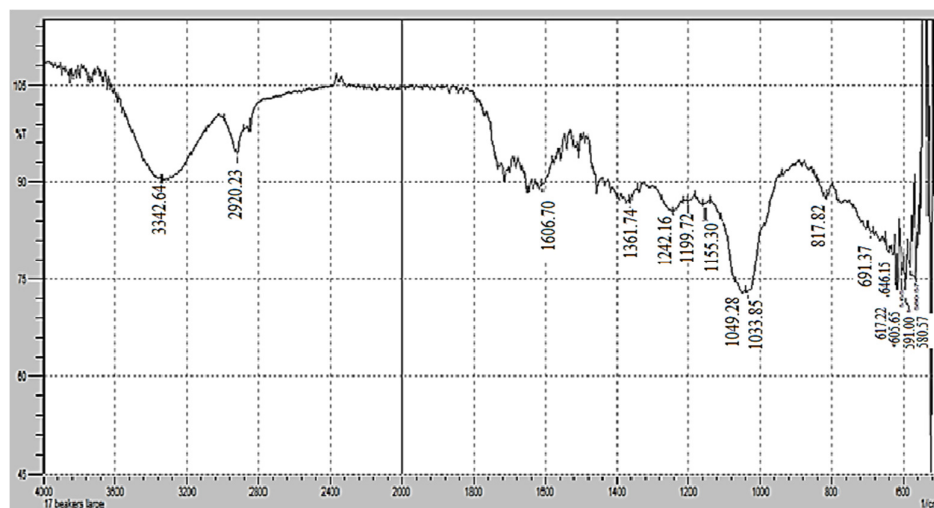


Figure 1. Neem leaves methanol extract FTIR spectra

FTIR spectra of methanol neem seed extract is shown in Figure 2. There are total 16 different peaks. These spectra are 3356.14 cm⁻¹, 2924.09 cm⁻¹, 2852.72 cm⁻¹, 1732.08 cm⁻¹, 1373.32 cm⁻¹, 1361.74 cm⁻¹, 1238.30 cm⁻¹, 1151.50 cm⁻¹, 1028.06 cm⁻¹, 991.41 cm⁻¹, 927.76 cm⁻¹, 817.82 cm⁻¹, 773.46 cm⁻¹, 709.80 cm⁻¹, 601.79 cm⁻¹ and 524.00 cm⁻¹.

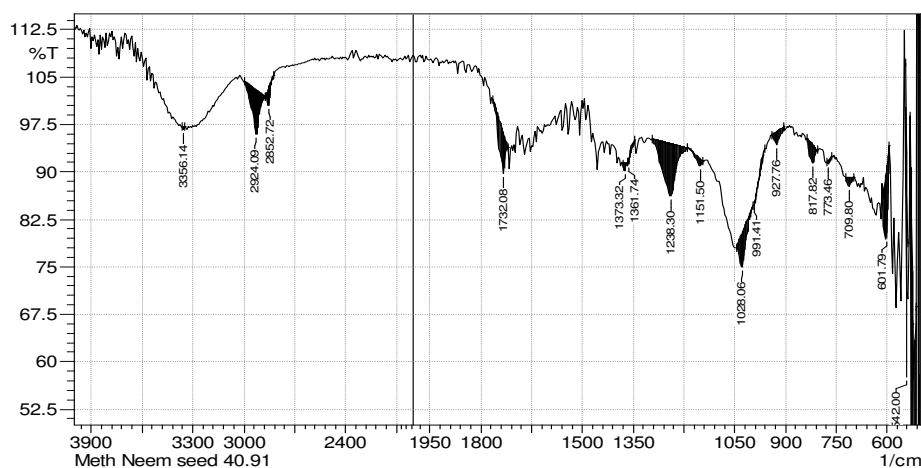


Figure 2. FTIR spectra of *A. indica* seed methanol extract

In Figure 3 FTIR spectra of methanol neem twigs extract is given. The total numbers of spectra are seven; which are 3350.35 cm^{-1} , 2926.01 cm^{-1} , 1722.43 cm^{-1} , 1454.33 cm^{-1} , 1382.96 cm^{-1} , 1236.37 cm^{-1} and 1051.20 cm^{-1} .

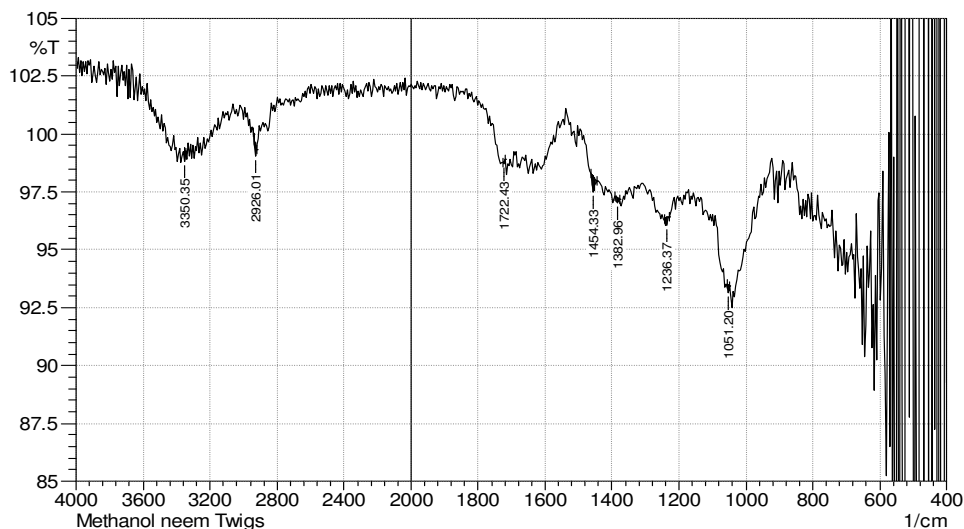


Figure 3. FTIR spectra of *A. indica* twigs methanol extract

FTIR spectra of chloroform neem leaves extract is displayed in Figure 4. There is a of total ten (10) spectra. These spectra are 2918.30 cm^{-1} , 2848.00 cm^{-1} , 1778.37 cm^{-1} , 1732.00 cm^{-1} , 1456.28 cm^{-1} , 1337.32 cm^{-1} , 1240.23 cm^{-1} , 1163.00 cm^{-1} , 1023.00 cm^{-1} and 719.43 cm^{-1} .

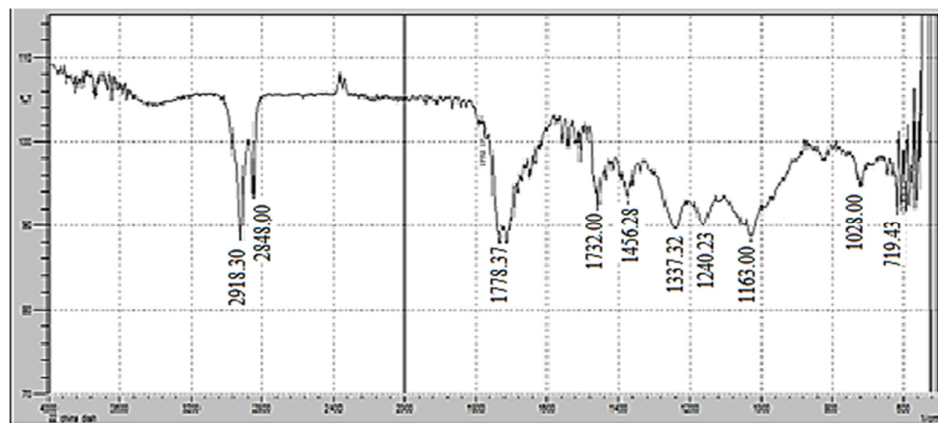


Figure 4. FTIR Spectra of *A. indica* leaves chloroform extract

FTIR spectra of chloroform neem seed extract is shown in Figure 5. There are ten (10) spectra appeared in the chromatogram. These spectra are 3334.92 cm^{-1} , 2922.16 cm^{-1} , 2852.72 cm^{-1} , 1737.86 cm^{-1} , 1558.48 cm^{-1} , 1456.20 cm^{-1} , 1240.23 cm^{-1} , 1161.15 cm^{-1} , 1023.85 cm^{-1} and 719.45 cm^{-1} .

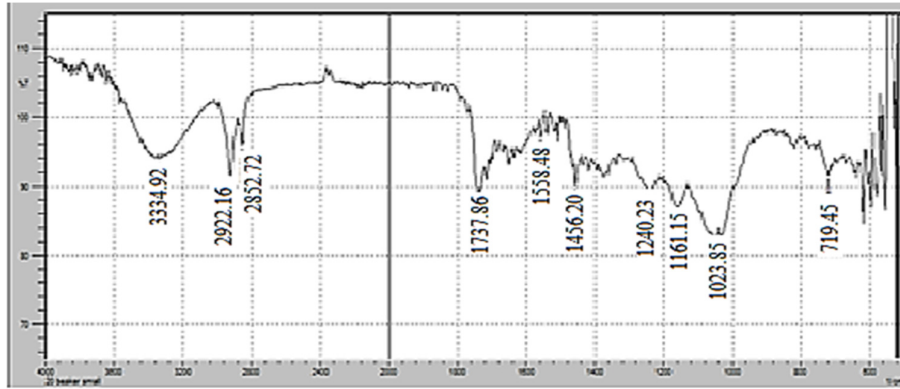


Figure 5. FTIR spectra of *A. indica* seeds chloroform extract

FTIR spectra of chloroform neem twigs extract is presented in Figure 6. There are ten (10) spectra appear in the chromatogram. These spectra are 2918.30 cm⁻¹, 2848.86 cm⁻¹, 1737.86 cm⁻¹, 1716.65 cm⁻¹, 1462.04 cm⁻¹, 1377.17 cm⁻¹, 1236.30 cm⁻¹, 1163.00 cm⁻¹, 1028.00 cm⁻¹ and 970.19 cm⁻¹.

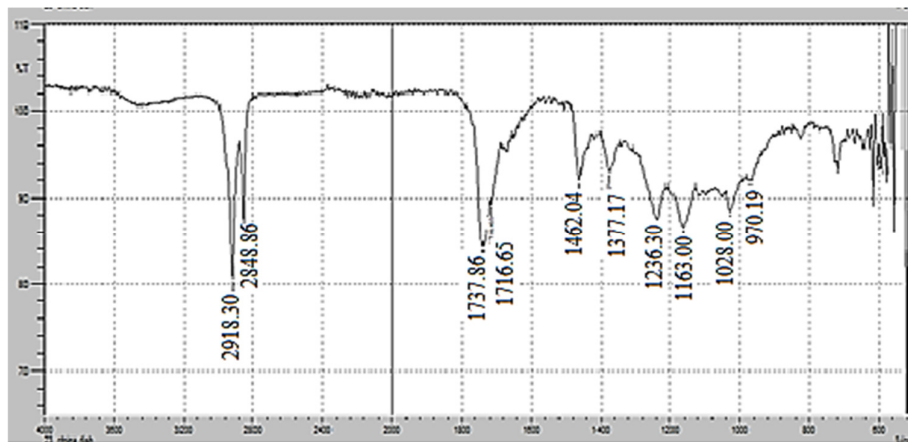


Figure 6. FTIR spectra of *A. indica* twigs chloroform extract

FTIR spectra for n-Hexane neem leaves extract is shown in Figure 7. The spectra shown twelve (12) peaks i.e. 2916.37 cm⁻¹, 2848.86 cm⁻¹, 1714.72 cm⁻¹, 1456.26 cm⁻¹, 1244.09 cm⁻¹, 1153.43 cm⁻¹, 1083.99 cm⁻¹, 1028.06 cm⁻¹, 1018.41 cm⁻¹, 993.34 cm⁻¹, 719.45 cm⁻¹ and 601.79 cm⁻¹.

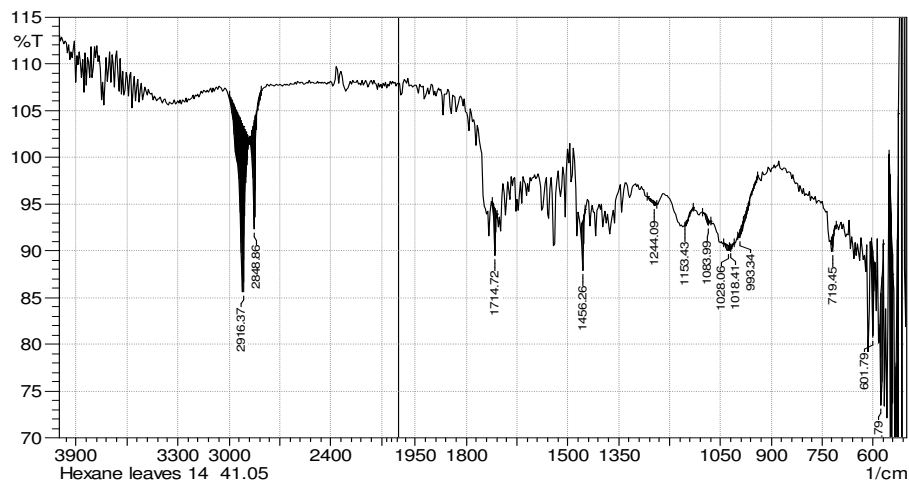


Figure 7. FTIR spectra of *A. indica* leaves n-Hexane extract

FTIR spectra of n-Hexane neem seed extract is shown in Figure 8. The chromatogram has five spectra i.e. 2922.16 cm^{-1} , 1743.65 cm^{-1} , 1234.44 cm^{-1} , 1161.15 cm^{-1} and 574.79 cm^{-1} . FTIR spectra of n-Hexane neem twigs extract is given in Figure 9, these spectra are 3381.21 cm^{-1} , 1647.21 cm^{-1} and 1016.49 cm^{-1} .

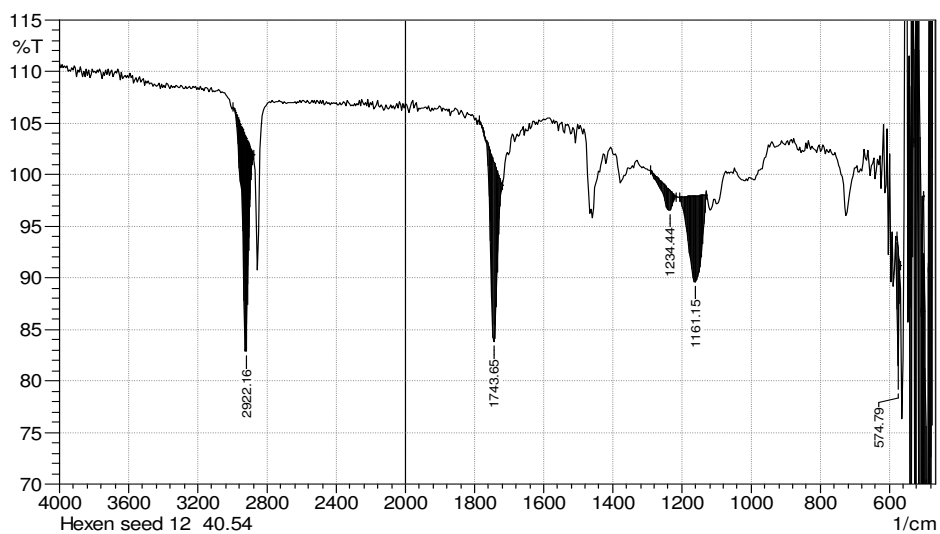


Figure 8. FTIR spectra of *A. indica* seed n-Hexane extract

Figure 9 shows FTIR spectra of *neem* twigs n-Hexane extract. The given chromatogram has three main peaks. These peaks are 3381.21 cm^{-1} , 1647.21 cm^{-1} and 1016.49 cm^{-1} respectively.

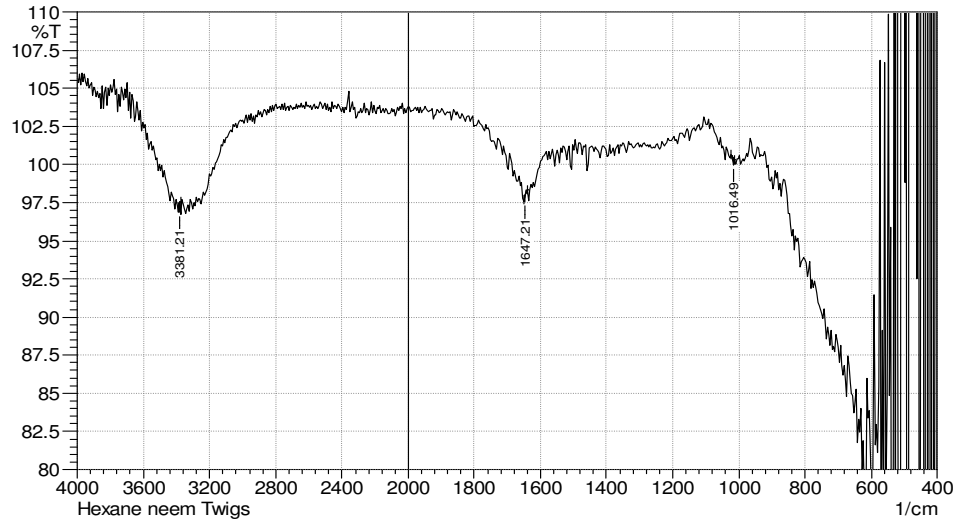


Figure 9. FTIR spectra of neem twigs n-Hexane extract

Figure 10 depicts FTIR spectra of ethanolic leaves extract of neem. The chromatogram shown sixteen (16) peaks i.e. 3332.99 cm^{-1} , 2931.80 cm^{-1} , 1714.72 cm^{-1} , 1608.63 cm^{-1} , 1361.74 cm^{-1} , 1338.60 cm^{-1} , 1311.59 cm^{-1} , 1242.16 cm^{-1} , 1199.72 cm^{-1} , 1049.26 cm^{-1} , 1033.65 cm^{-1} , 817.62 cm^{-1} , 775.38 cm^{-1} , 667.37 cm^{-1} , 619.15 cm^{-1} and 605.65 cm^{-1} .

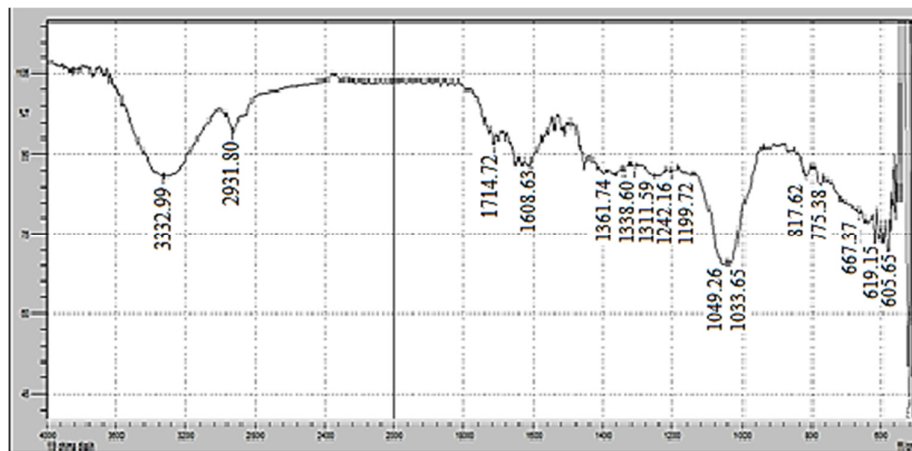


Figure 10. FTIR Spectra of *A. indica* leaves ethanol extract

Figure 11 shows FTIR spectra of ethanol neem seed extract. In the chromatogram there are eleven (11) peaks i.e. 3323.36 cm^{-1} , 2918.30 cm^{-1} , 2848.86 cm^{-1} , 1732.08 cm^{-1} , 1446.61 cm^{-1} , 1244.09 cm^{-1} , 1155.36 cm^{-1} , 1049.28 cm^{-1} , 1035.77 cm^{-1} , 719.45 cm^{-1} and 663.51 cm^{-1} .

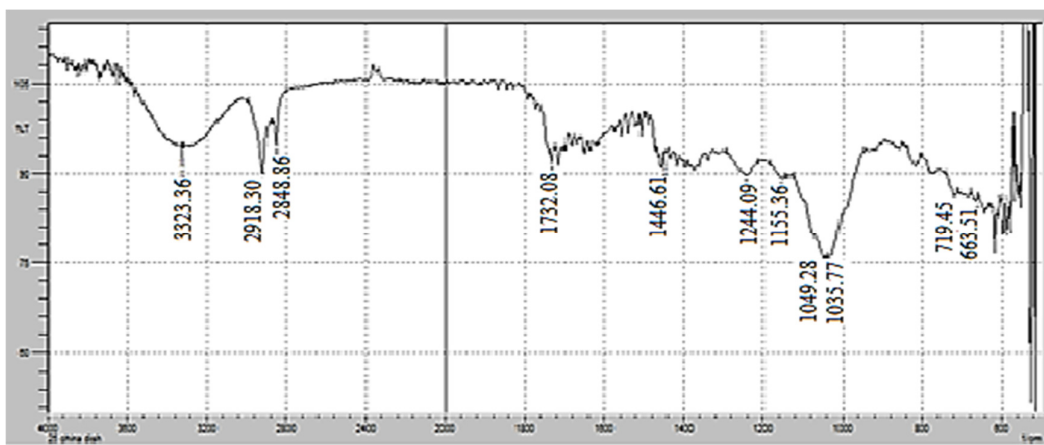


Figure 11. FTIR spectra of *A. indica* seed ethanol extract

FTIR spectra of ethanol neem stem extract is shown with twelve peaks in Figure 12. These peaks are 3354.21 cm⁻¹, 2922.16 cm⁻¹, 2852.72 cm⁻¹, 1732.06 cm⁻¹, 1456.26 cm⁻¹, 1373.32 cm⁻¹, 1238.30 cm⁻¹, 1157.29 cm⁻¹, 1049.26 cm⁻¹, 1029.99 cm⁻¹, 709.80 cm⁻¹ and 599.86 cm⁻¹.

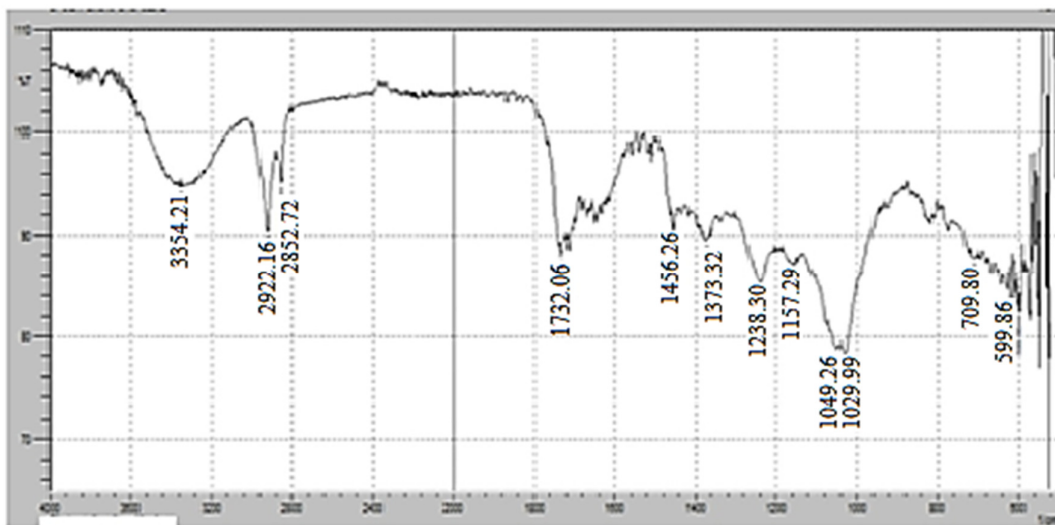


Figure 12. FTIR spectra of *A. indica* twigs ethanol extract

Figure 13 depicts FTIR spectra of ethyl acetate neem leaves extract is shown ten (10) peaks. These spectra are 2918.30 cm⁻¹, 2848.00 cm⁻¹, 1732.06 cm⁻¹, 1714.72 cm⁻¹, 1456.26 cm⁻¹, 1373.32 cm⁻¹, 1240.23 cm⁻¹, 1165.00 cm⁻¹, 1028.00 cm⁻¹ and 719.45 cm⁻¹.

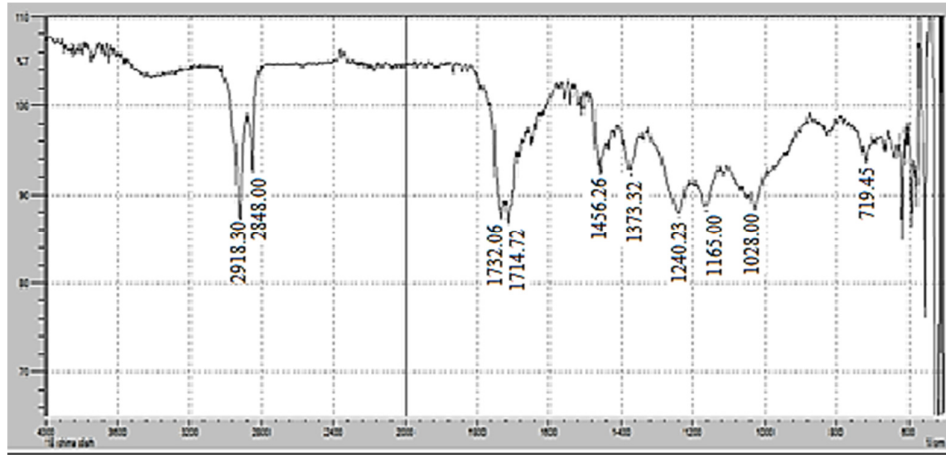


Figure 13. FTIR spectra of *A. indica* leaves ethyl acetate

FTIR spectra of ethyl acetate neem seed extract is presented in Figure 14 with seven peaks. These peaks are 2922.16 cm⁻¹, 2852.72 cm⁻¹, 1739.79 cm⁻¹, 1456.26 cm⁻¹, 1238.30 cm⁻¹, 1161.15 cm⁻¹ and 721.36 cm⁻¹.

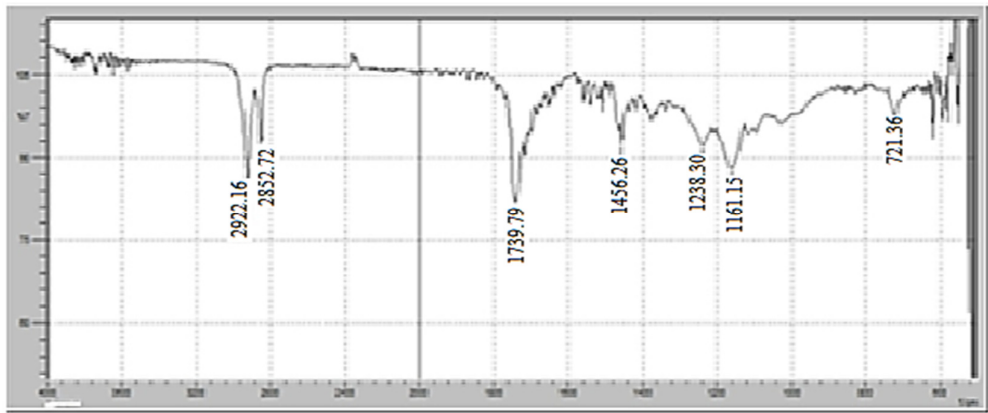


Figure 14. FTIR spectra of *A. indica* seed ethyl acetate

FTIR spectra of ethyl acetate neem twigs extract is shown in Figure 15 having 10 peaks. These peaks are 2918.30 cm⁻¹, 2848.86 cm⁻¹, 1737.86 cm⁻¹, 1556.46 cm⁻¹, 1456.26 cm⁻¹, 1373.32 cm⁻¹, 1238.30 cm⁻¹, 1163.08 cm⁻¹, 1028.00 cm⁻¹ and 719.45 cm⁻¹.

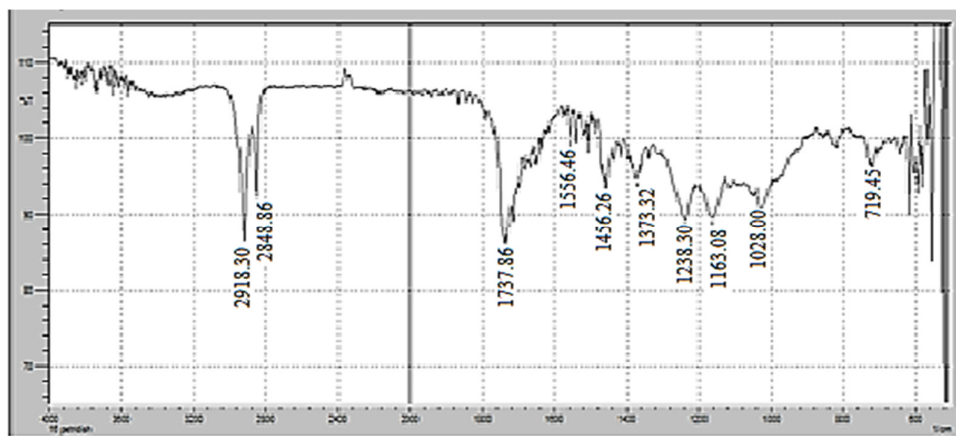


Figure 15. FTIR spectra of *A. indica* twigs ethyl acetate

The FTIR spectra of neem leaves powder displayed a peak value 3423 cm^{-1} is accredited to stretching vibration of the functional group (O-H) for an intramolecular H bond or a group of phenol in leaves of neem. The sharp stretching peak is also attributed for bending vibration of amine group (NH_2) and N-H stretching (Oyekanmi *et al.*, 2021). The band peak value 2924 cm^{-1} accredited to C-H stretching vibration modes in the hydrocarbon chain, whereas the absorption peak at 1734 cm^{-1} corresponding to the stretching vibrations of the functional group (carbonyl) in carboxylic acids, aldehydes and ketones (Kowalonek, 2007). The peak value 1641 cm^{-1} is credited to C=O stretching amide designating the occurrence of group $-\text{COOH}$ in the leaves of neem and the spectra at 1384 cm^{-1} match to the stretching vibrations that sourced from C-H stretching, CH_2 wagging, C=O and C-O bonds of acetyl esters respectively. The aliphatic C-C position was observed at 1249 cm^{-1} and powder neem leaves the peak at 1070 cm^{-1} was given to C-O stretching vibration (Oyekanmi *et al.*, 2021). The FTIR spectra of chemical class of compounds i.e heterocyclic compound were 1509 cm^{-1} , 1266 cm^{-1} , 1090 cm^{-1} , 724 cm^{-1} , 606 cm^{-1} , flavonoids expected FTIR spectra 1720 cm^{-1} , 1655 cm^{-1} , n-acyl amines expected FTIR spectra 3366 cm^{-1} , 3164 cm^{-1} , 2962 cm^{-1} , 1662 cm^{-1} , 1634 cm^{-1} , 1430 cm^{-1} , 1418 cm^{-1} , 1263 cm^{-1} and phenolic compounds expected FTIR spectra were 3343 cm^{-1} , 3168 cm^{-1} , 1671 cm^{-1} , 1618 cm^{-1} , 1414 cm^{-1} , 1398 cm^{-1} (Guchhait *et al.*, 2022).

Carboxylic acids hold a carbonyl class and alcohol group which accounts numerous basics chemical and physical properties with ketones, aldehydes and alcohols. The prepared carboxylic acid esters were *In-vitro* assessed against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus niger*. The entire derivatives of carboxylic acid ester displayed maximum fungicidal activities as compared to standard fluconazole (Sadiquallah *et al.*, 2018). Aldehyde is a biological molecule having a formyl group. Amongst the aldehyde, glutaraldehyde is a vital member utilized as sterilant and disinfectant. Glutaraldehyde has a broad range of activities against fungi, bacteria and virus. Formaldehyde and cinnamaldehyde are used as fungistatic. The aromatic halogenated dinitriles exhibited significant biological potentials like bactericides, fungicides and nematocides (Sadiquallah *et al.*, 2018).

Conclusions

The results of the study showed that neem leaves and seeds extracts have significant fungicidal potential as evident from significant inhibition zones observed against tested plant pathogenic fungi. The FTIR spectroscopic analysis have confirmed different functional groups identifying the presence of various phytochemical groups in *A. indica* twigs, seeds and leaves extracts that were responsible for the fungicidal

properties recorded. Based on the obtained results it was concluded that *A. indica* if used as biopesticide would be a greener and better alternative to synthetic fungicides applied in postharvest in the management of plant's fungal diseases caused by *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Penicillium expansum*. Plant origin biopesticides may be a foundation of innovative substitute of the synthetic fungicides as they are cheaper, ecofriendly and non-toxic in nature. Further studies are needed in order to determine the underlying molecular mechanism of control and to reveal the neem component accountable in controlling these tested pathogens. The research further suggests field studies trails for diverse crops, fruits and vegetable to control the plant pathogenic fungi.

Authors' Contributions

Conceptualization: JA; Data curation: AS and MS; Formal analysis: NG and ABS; Funding acquisition: RU and MAI; Investigation: AS; Methodology: JA; Project administration: JA; Resources: JA; Software: AS; Supervision: JA; Validation: MI and NG; Visualization: RU and NG; Writing - original draft JA, AS, MS, MI and MZ; Writing - review and editing: MS, MI and MZ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abid HQ, Sajad AM, Jamaluddin, Shoket A (2016). Phytochemical analysis and antifungal activities of *Azadirachta indica* against different fungi isolated from infected Solanaceous vegetable fruits. International Journal of Advanced Research 4(12):1626-1630. <https://doi.org/10.21474/IJAR01%2F2564>
- Ali J, Abbas J (2023). Comparative bioactive compounds and antifungal potential evaluation of *Mentha longifolia* (L.) Hudson (Lamiaceae) extracts against plant pathogenic fungi. International Journal of Environmental Science and Technology 15(2):48-57. <http://dx.doi.org/10.4314/ijest.v15i2.5>
- Alzohairy MA (2016). Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. Evidence Based Complementary Alternative Medicine 2016:1-11. <https://doi.org/10.1155/2016/7382506>
- Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, Okebugwu P (2011). Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. Nature and Science of Sleep 9(9):53-61.
- Anthony KO (2000). Antifungal effect of *Azadirachta indica* A. Juss extracts on *Colletotrichum lindemuthianum*. Global Journal of Pure and Applied Sciences 6(3):425-478.

- Awache I, Gero M, Yakubu MN, Ubandoma A, Yusuf A, Acha M (2017). Phytochemical properties and the *in-vitro* antibacterial activity of neem (*Azadirachta indica*) twigs extract on bacteria isolated from human mouth. Health Science Research 4(6):68-71.
- Benisheikh AAG, Fatimah MM, Harun K, Zainab MA, Umar BM, Mallam WJ (2019). Phytochemical extraction and antimicrobial studies on crude leaf extract of *Azadirachta indica* (Neem) in semi-arid region of Borno State, Nigeria. International Journal of Research and Review 6(12):516-522.
- Bharat P, Sagar R, Sulav R, Ankit P (2015). Investigations of antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. African Journal of Biotechnology 14(46):3159-3163. <https://doi.org/10.5897/AJB2015.14811>
- Bhatnagar D and McCormick SP (1988). The inhibitory effect of neem (*Azadirachta indica*) leaf extracts on aflatoxin synthesis in *Aspergillus parasiticus*. Journal of the American Oil Chemists' Society 1988 65(7):1166-1168. <https://doi.org/10.1007/BF02660575>
- Bhatnagar D, Zeringue HJ (1993). Neem leaf extracts (*Azadirachta indica*) inhibit biosynthesis in *A. flavus* and *A. parasiticus*. Proceedings of the World *Neem* Conference, Bangalore, India. pp 24-28.
- Dash SP, Dixit S, Sahoo S (2017). Phytochemical and biochemical characterizations from leaf extracts from *Azadirachta indica*: an important medicinal plant. Biochemistry and Analytical Biochemistry 6(323):2161-1009. <http://dx.doi.org/10.4172/2161-1009.1000323>
- Dauda H, Murtala NG, and Bulama A (2015). Use of neem (*Azadirachta indica*) seed powder to treat groundnut seed-borne pathogenic fungi. European Journal of Experimental Biology 5(5):69-73.
- de Oliveira Filho JG, Silva GD, Cipriano L, Gomes M, Egea MB (2021). Control of postharvest fungal diseases in fruits using external application of RNAi. Journal of Food Science 86(8):3341-8. <https://doi.org/10.1111/1750-3841.15816>
- Effiong EB, Gwana AM, Halima MB, Umeh SO, Bagudu BY, Okoli CM (2016). Phytochemical analysis and antimicrobial activity of methanolic, ethanolic and acetonitrilic extracts of stem bark and leaf of neem plant (*Azadirachta indica*). Journal of Diseases and Medicinal Plants 2(3):14-25. <https://doi.org/10.11648/j.jdmp.20160203.11>
- El-Hawary SS, El-Tantawy ME, Rabeh MA, Badr WK (2013). DNA fingerprinting and botanical study of *Azadirachta indica* A. Juss. (neem) family Meliaceae. Beni-Suef University Journal of Basic and Applied Sciences 2(1):1-13. <http://dx.doi.org/10.1016/j.bjbas.2013.09.001>
- Elisa B, Giuliano B, Vincenzo A, Behzad Z, Ebrahim S, Felice S, Virginia L (2007). Saponins from *Allium minutiflorum* with antifungal activity. Phytochemistry 68(5):596-603. <https://doi.org/10.1016/j.phytochem.2006.10.009>
- El-Mahmood AM, Ogbonna OB, Raji M (2010). The antibacterial activity of *Azadirachta indica* (neem seeds extracts) against bacterial pathogens associated with eye and ear infections. Journal of Medicinal Plants Research 4(14):1414-1421.
- Evbuomwan BO, Felix-Achor I, Opute CC (2015). Extraction and characterization of oil from Neem seeds, leaves and barks. European International Journal of Science and Technology 24(7):1-7.
- Ghimeray AK, Jin C, Ghimire BK, Cho DH (2009). Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal. African Journal of Biotechnology 8(13):3084-3091.
- Govindachari TR, Suresh G, Gopalakrishnan G, Banumathy B, Masilamani S (1998). Identification of antifungal compounds from the seed oil of *Azadirachta indica*. Phytoparasitica 26(2):109-116. <https://doi.org/10.1007/BF02980677>
- Guchhait KC, Manna T, Barai M, Karmakar M, Kumar NS, Jana D, ... Ghosh C (2022). Antibiofilm and anticancer activities of unripe and ripe *Azadirachta indica* (neem) seed extracts. BMC Complementary Medicine and Therapies 22(1):42. <https://doi.org/10.1186/s12906-022-03513-4>.
- Imam H, Azad H, Makbul S (2012). Neem (*Azadirachta indica* A. Juss)-A nature's drugstore: An overview. International Research Journal of Biological Sciences 1:76-79.
- Jessinta DOS, Azhari HN, Saiful NBT and Abdurahman HN (2013). Fatty acid composition and antibacterial activity of Neem (*Azadirachta indica*) seed oil. Open Conference Proceedings Journal 4:43-48.

- Joshi M, Purwar R, Ali SW, Rajendran S (2010). Antimicrobial textiles for health and hygiene applications based on eco-friendly natural products. In: Medical and Healthcare Textiles. Elsevier pp 84-92.
- Khajista J (2013). Antifungal activity of *Azadirachta indica* against *Alternaria solani*. Journal of Life Sciences and Technology 1(1):89-93. <https://doi.org/10.12720/jolst.1.1.89-93>
- Kowalonek J (2017). Studies of chitosan/pectin complexes exposed to UV radiation. International Journal of Biological Macromolecules 103:515-524. <https://doi.org/10.1016/j.ijbiomac.2017.05.081>
- Mahmoud D, Hassanein N, Youssef K, Abou Zeid M (2011). Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. Brazilian Journal of Microbiology 42(3):1007-16. <https://doi.org/10.1590/s1517-838220110003000021>
- Mondali NK, Mojumdar R, Chatterje SK, Banerjee A, Datta JK, Gupta S (2009). Antifungal activities and chemical characterization of neem leaf extracts on the growth of some selected fungal species *in vitro* culture medium. Applied Sciences and Environmental Management 13(1):49-53.
- Narasimharaju K, Nagarasanakote VT, Nagepally VJ, Ramaiah N, Sathyanarayana S, Bharat K (2015). Antifungal and antioxidant activities of organic and aqueous extracts of *Annona squamosal* Linn. Leaves. Journal of Food and Drug Analysis 23:795-802. <https://doi.org/10.1016/j.jfda.2015.04.012>
- Norazlina H, Suhaila A, Lili SH, Saidatul RG, Rafidah J (2021). A study of neem leaves: Identification of method and solvent in extraction. Materials Today: Proceedings 42:217-221. <http://dx.doi.org/10.1016/j.matpr.2020.11.726>
- Osborn AC (1996). Saponins and plant defence—a soap story. Trends in Plant Sciences 1:4-9. [https://ui.adsabs.harvard.edu/link_gateway/1996TPS.....1....4O/doi:10.1016/S1360-1385\(96\)80016-1](https://ui.adsabs.harvard.edu/link_gateway/1996TPS.....1....4O/doi:10.1016/S1360-1385(96)80016-1)
- Ospina Salazar DI, Hoyos Sanchez RA, Orozco Sanchez F, Arango Arteaga M, Gomez Londono LF (2015). Antifungal activity of neem (*Azadirachta indica*: Meliaceae) extracts against dermatophytes. Acta Biológica Colombiana 20(3):181-92. <http://dx.doi.org/10.15446/abc.v20n3.45225>
- Oyekanmi AA, Kumar USU, HPS AK, Olaiya NG, Amirul AA, Rahman AA, ... Rizal S (2021). Functional properties of antimicrobial neem leaves extract based macroalgae biofilms for potential use as active dry packaging applications. Polymers 13(10):1664-1670. <https://doi.org/10.3390/polym13101664>
- Qasem JR, Abu-Blam HA (1996). Fungicidal activity of some common weed extracts against different plant pathogenic fungi. Journal Phytopathology 144:157-161. <https://doi.org/10.1111/j.1439-0434.1996.tb01507.x>
- Saba JG, Firas MAS, Ragheb AT (2010). Antifungal activity of several medicinal plants extracts against the early blight pathogen (*Alternaria solani*). Archives of Phytopathology and Plant Protection 43(17):1745-1757. <https://doi.org/10.1080/03235401003633832>
- Sadiqullah K, Muhammad IS, Abdul H, Khawaja WA, Tabassum RMB, Said AK (2018). Fourier-Transform Infrared Spectroscopy analysis and antifungal activity of methanolic Extracts of *Medicago parviflora*, *Solanum nigrum*, *Melilotus alba* and *Melilotus indicus* on soil-borne Phytopathogenic fungi. Pakistan Journal of Botany 50(4):1591-1598.
- Samuel KT, Francis A, John A, Enoch A (2021). Potential of neem extracts as natural insecticide against fall armyworm (*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Case Studies in Chemical and Environmental Engineering 4:100130. <https://doi.org/10.1016/j.csee.2021.100130>
- Saniewska A, Bialy Z, Jurzysta M, Jarecka A (2005). Antifungal activity of saponins from *Medicago arabica* L. shoots against some pathogens. Allelopathy Journal 16(1):105-111.
- Sharma N, Tripathi A (2006). Fungitoxicity of the essential oil of *Citrus sinensis* on postharvest pathogens. World Journal of Microbiology and Biotechnology 22(6):587-593. <https://doi.org/10.1007/s11274-005-9075-3>
- Sheema KK, Durai M (2014). Fungicidal activity of aqueous leaf extract on *Alternaria brassicae*. International Journal of Pharmacognosy and Phytochemical Research 6(4):801-805.
- Shuaibu I, Hamman JB, Goje LJ, Muinat AM, Jauro HA, Kabiru MY (2015). Phytochemical analysis and evaluation of bacteriostatic effect of neem (*Azadirachta indica*) leaves on some clinical bacterial isolates. Journal of Harmonized Research in Applied Science 3(3):152-157.
- Sprag SG, Light ME, Van SJ (2004). Biological activities and distribution of plant saponins. Journal Ethnopharmacology 94:218-243. <https://doi.org/10.1016/j.jep.2004.05.016>
- Srivastava A, Shukla Kumar YN (2000). Recent development in plant derived antimicrobial constituents A Review. Journal of Medicinal and Aromatic Plant Sciences 20:717-72.

- Sultana B, Anwar F, Ashraff M (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 14:2167-2180. <https://doi.org/10.3390/molecules14062167>.
- Tesfaye B, and Tefera T (2021). Extraction of essential oil from seed by using Soxhlet extraction methods. *International Journal of Advances in Engineering and Management Sciences* 3(6):646-650. <https://dx.doi.org/10.24001/ijaems.3.6.5>
- Usha K, Singh B, Praseetha P, Deepa N, Agarwal DK, Agarwal R and Nagaraja A (2009). Antifungal activity of *Datura stramonium*, *Calotropis gigantea* and *Azadirachta indica* against *Fusarium mangiferae* and floral malformation in mango. *European Journal of Plant Pathology* 124 (4):637-657. <https://doi.org/10.1007/s10658-009-9450-2>.
- VanEtten HD, Mansfield JW, Bailey JA, Farmer EE (1994). Two classes of plant antibiotics: phytoalexins versus phytoanticipins. *Plant Cell* 6(9):1191-1192. <https://doi.org/10.1105/tpc.6.9.1191>.
- Wafaa AH, Howaida IAA, Hassan A and El-Safty MM (2007). Chemical composition and *in vitro* antiviral activity of *Azadirachta indica* A. Juss (Neem) leaves and fruits against Newcastle disease virus and infectious Bursal disease virus. *Australian Journal of Basic and Applied Sciences* 1(4):801-812.
- Yang CR, Zhang Y, Jacob MR, Khan SI, Zhang YJ, Li XC (2006). Antifungal activity of C-27 steroidal saponins. *Antimicrobial Agents and Chemotherapy* 50(5):1710-1714. <https://doi.org/10.1128/aac.50.5.1710-1714.2006>



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