ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VII July 2023

Phytochemical Screening, Antioxidant and Antimicrobial Activities of Neem Seed (*Azadirachta indica)* Extracts

Awode, A. U., Ezera, J. E., Adoga, S. O, and Wapwera, J. A

Department of Chemistry, Faculty of Natural Sciences, University of Jos, Nigeria

DOI: https://doi.org/10.51584/IJRIAS.2023.8704

Received: 28 May 2023; Accepted: 27 June 2023; Published: 28 July 2023

Abstract: In this study, the phytochemical, antimicrobial and antioxidant activity of neem seed oils (*Azadirachta indica)* was analyzed. The extract was extracted by solvent extraction using n-hexane, ethyl acetate, methanol and aqueous solvents. The percentage yields of the extraction were 42.50%, 40.70%, 38.30% and 28.50% for the n-hexane, ethyl acetate, methanol and aqueous solvents respectively. The phytochemical screening of the samples revealed the presence of alkaloids, flavonoids, steroids, anthraquinones, cardiac glycosides and terpenoids in neem seed extract. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration of the neem seed extract was determined *on Escherichia coli, Staphylococcus aureus, Aspergillus niger and Candida albicans*. The MIC for neem seed extract in methanolic extract on *E. coli* and S. *aureus* was the least at 6.25% concentration while the MIC on the fungi (*A. niger*) was at 50% concentration having a zone of inhibition of 7.67± 0.71mm. There was no growth inhibition in *C. albicans.* The neem seed extract was shown to possess an antioxidant activity using DPPH radical. There was a significant increase in the scavenging activity of the neem seed extract as the concentration increased from 6.25% to 100%. The blended quantity of the neem seed extract showed the highest scavenging activity of 54.19 ± 0.03%. The study shows the extracts of neem seed possess good bioactive agents, antioxidant and antibacterial activity, and therefore they could be effectively used as a natural source of antioxidants and to be detected against gram-positive bacteria.

Keywords: Phytochemical screening; Antioxidants; Antimicrobial; DPPH; Terpenoids; Neem seed extracts; Minimum Inhibitory Concentration (MIC); Minimum Bactericidal Concentration (MBC).

I. Introduction

Medicinal plants are important species of plants that according to the traditional medicinal practices and also from modern scientific studies are useful for medicinal purposes to alleviate diseases, make human health more invigorating. These plants are contemplated as rich sources of ingredients that can be used in the synthesis and production of drugs (Oladeji *et al.*, 2019). Plants consist of various kinds of chemical constituents known as phytoconstituents, they serve the plants by contributing some secondary functions like; helps in plant growth, safeguarding the plants by activating defense mechanism, imparting color, odor, and flavor to the plants (Molyneux et al., 2007). Natural products and their derivatives exhibit minimal side effects and improved efficacy than other synthetic counterparts. These plant-derived components like flavonoids, quinine, terpenoid etc conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties (Batiha et al., 2020).

Neem tree (*Azadirachta indica*) is a member of the mahogany family (*Meliaceae)* and endemic to the Indian subcontinent (Liauw *et al.*, 2008). Neem tree proliferates in the tropical and semi-tropic climate countries, including Nigeria where it is known as *Dongoyaro,* with good environmental adaptability. The parts of the neem plant like leaves, barks, flowers, fruits, seeds, and roots were good sources of native medicine for the household treatment of various human illnesses and industrial products (Tesfaye *et al.*, 2018). The neem seed has the highest concentration of oil compared to other parts of the tree and this oil is used as lubricants, insecticides, and drugs for a variety of diseases like; diabetes, leprosy, and tuberculosis (Tesfaye *et al.*, 2018). Neem seed oil is also used in the manufacturing of a large number of skin products such as body lotions, body soaps, and beauty cares facial packs in combination with other natural ingredients.

While the cake that remains after the oil extraction serves as an active ingredient in the manufacturing of mosquito repellent coils (Liauw *et al.*, 2008). Although neem seed oils are not majorly used for cooking purposes because of their offensive odour and bitter taste, similar to the combined odours of garlic and peanut, however, it is used as a preservative agent to prolong the shelf-life of cowpea grains (Ilesanmi and Gungula, 2011). Thus the increase in the production, characterization, and utilization of this seed in vegetable oils to meet global needs.

It was demonstrated that neem seeds contain a large plethora of constituents, which influence the biological activity. In terms of fatty acid composition, this commercial neem oil is mainly composed of oleic (58%), palmitic (14%) and stearic (15%)

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VII July 2023

acids, whereas myristic, arachidic, linoleic and behenic acids are detectable only in small amounts. In the obtained oil, triterpenoids such as salannin, nimbin and azadirachtin, known for their insecticide properties, are also present along with sterols. All these molecules are recognized as biologically active ingredients (Pandey et al., 2014). Nimbin, accounting for much of the biological activities of Neem oil, shows anti-inflammatory, antipyretic, fungicidal, antihistamine and antiseptic properties (Gupta et al., 2017). Azadirachtin, a powerful acetylcholinesterase inhibitor (Nathan et al. 2008), and salannin are undergoing evaluation for their inhibitory activity (Akihisa et al., 2017).More than 140 compounds have been isolated from all parts of the Neem tree (flowers, leaves, seeds, fruits, roots, bark), to which interferon inducing activity (bark), immunomodulatory, antipyretic and anti-inflammatory, antiulcer, antimalarial, antifungal, antibacterial, antiviral against skin ailments activity (leaves), as well as antioxidant and anti-mutagenic properties were recognized (SaiRam et al., 2000).

There is an increasing trend in research for medicinal plants which contain bioactive compounds such as phytochemicals, antioxidants, antimicrobials in plant extracts which are used in designing functional foods and drugs which prevent diseases and improve the quality of human health. This is due to the increase in antimicrobial resistance activities in drugs which is a threat to public health, hence the need to analyze extracts of Neem seed for the purpose of quality food and drug development.

There is a more growing trend in searching for Phytochemicals, antioxidants of natural origin. Fresh fruits are an excellent source of natural antioxidants and some of them even outperform the synthetic antioxidants, and are safer also from the health point of view.

Fig 1: Fresh neem seed

Traditional use of neem extract as herbs has shown that benefits exist when consumed (Yerima et al., 2012). Therefore, the need for identification bioactive components of the plant extract and its activities through phytochemical, antioxidant and antimicrobial analysis.

This research work is aimed at studying the phytochemical, antioxidant and antimicrobial constituents of neem seed extract.

II. Materials and Methods

Apparatus/Equipment

Weighing scale, electric mixer, microwave, digital thermometer, spatula, beakers, digital pH meter, Lab equipment, testing equipment. All the equipment were washed and rinsed with distilled water and dried in an oven before use. This was done to avoid any contamination and interference with the reagents.

Reagents

Mayer's reagent, Dragendorff's reagent, Picric reagent, Ferric chloride, Gentamicin, Sulphuric acid, Acetic anhydride, Dilute Ammonia, 1,1-diphenyl-2-picrylhdrazyl (DPPH), Distilled water, methanol, n-hexane and Ethyl acetate, Resazurin dye.

Source of Microorganisms.

Standard isolates of the bacteria *E. coli* and *S.aureus* and fungi *Aspergillus niger,* and *C.albicans*were obtained from the Veterinary Research Institute Vom, Jos-south Local Governemt area of Plateau state, Nigeria. The organisms were collected in suspension of sabouraud broth.

Collection of Samples

The Neem seeds were collected from of the local sources in Jos, plateau state. The samples were washed with water to remove dust and foreign particles then shade dried.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VII July 2023

Preparation of Neem Seed Oil extracts

Four parts each of 40 g of Neem seeds were weighed and put into the thimble of the Soxhlet extractor which was connected to a condenser unit and water source for cooling rising solvent vapour. Each part of the Neem seed was soaked in water, methanol, n-hexane and Ethyl acetate (Sofowora 2008). The percentage yield of each extract was determined.

Phytochemical Determination

a) Test for Alkaloids

About 0.5g of each extract was stirred with 3ml of 1% aqueous hydrochloric acid on a steam bath; 1ml each of the filtrate was treated with a few drops of Mayers reagent, Dragendorff's reagent and Picric solution.

Precipitation with either of this reagents was taken as preliminary evidence for the presence of alkaloid in the extract (Sofowora, 2008).

b) Test for Saponins

About 0.5g of each plant extract was shaken with water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins (Sahira-Banu and Cathrine 2015).

c) Test for Tannins

About 0.5g of plant extract was stirred with 1ml of distilled water and filtered, ferric chloride was added to the filtrate. A blue-black, green, or blue green precipitate indicated the presence of tannins (Sahira-Banu and Cathrine 2015).

d) Test for Anthraquinones

Borntrager's test was be used for the detection of anthraquinones, 0.5g of each extracts was taken into a dry test tube and 5ml of chloroform was added and shook for 5 minutes. The extract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of free authraquinones (Sahira-Banu and Cathrine 2015).

e) Test for Cardiac Glycoside

100 mg of the extract was dissolved in 70% alcohol and filtered. About 3 drops of lead sub- acetate was introduced into the filtrate and filtered. The filtrate was extracted with 10mls of choloform in a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1ml of glacial acetic acid containing one drop of Ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of a deoxysugar characteristic of cardenolides (Sahira-Banu and Cathrine 2015).

f) Test for Steroid and Terpenoides

A little quantity of each extract was dissolved in chloroform, and 1ml of acetic anhydride was added, then two drops of concentrated Sulphuric acid was added. A pink colour which changes to bluish green on standing is indicative of the presence of steroid and tepenes (Sahira-Banu and Cathrine 2015).

g) Test for Flavonoids

Dilute ammonia (5 ml) was added to 5 ml extract and then 5 ml concentrated sulfuric acid was added. Formation of yellow colour shows the presence of flavonoids (Sahira-Banu and Cathrine 2015).

h) Test for Carbohydrates

Each extract (100 ml)was dissolved in 3ml of distilled water and mixed with a few drops of Molisch reagent (10% solution of napthol in alcohol) then 1ml of concentrated sulphuric acid was carefully added down the side of the inclined tube so that the acid formed a layer beneath the solution. A white colour at the base indicated the presence of carbohydrates (Sofowora, 2008).

InvitroAntioxidant Activity Assay

The effect of fractions on DPPH radical was estimated. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this was mixed with 1.0 ml of different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25mg/ml) of the different fractions in methanol. The reaction mixture was vortexed thoroughly and left in the dark at room temperature 27º C for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. The ability to scavenge DPPH radical was calculated by the following equation (Liyana-Pathiranan and Shahidi, 2005):

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VII July 2023

Scavenging activity (%) = $\frac{(Absorbance\ of\ blank - Absorbance\ of\ samples)}{Absorbance\ of\ blank} \times 100$ (equation 1)

Antimicrobial Susceptibility Testing

Agar Well Diffusion Techniques

The antimicrobial susceptibility test was performed with the clinical isolate using the agar well diffusion technique. The bacteria inoculum was prepared from subculture as follows; colony of a day old bacteria was suspended in broth and turbidity adjusted to 0.5 McFarland standard. The sterile cotton wool swap method was used to inoculate the bacteria on solidify plates of nutrient agar. Similarly, fungal inoculum was prepared from subculture as follows; the spores of three day old fungi was suspended in broth and turbidity adjusted to 0.5 McFarland standard. The pour plate method was used to inoculate organism on SDA.10ml of broth containing spores was pour in petri dish and 20 ml 0f SDA was added and shake for even distribution and was allow to solidify. Wells of about 6mm in diameter were aseptically punched with a sterile cork borer (5 holes per plates) and the wells were filled with 100 micro liter of the different concentration of the plant extracts. The plates were left for 30 minutes before incubation in order for the extracts to diffuse into the agar. The plates were incubated at 37^0c for 24 hours and the zones of inhibition were measured to the nearest millimeter (mm) (Nair and Chanta (2005).

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC).

It was determine using the broth dilution method using the 96 micro titer plates using resazurin dye as growth indicator. Any significant change in colour from deep blue to purple colour was taken after 24 hours after incubation at 37°c as concentration for presence and absence of growth (Banfi et al., 2003).

III. Results and Discussion

Table 1: Percentage yield of the neem seed extract using n-Hexane, ethyl acetate, methanol and aqueous solvent.

Solvent	Yield (%)
n-Hexane	50.50
Ethyl acetate	45.00
Methanol	38.30
Aqueous	35.50

Table 1 is the percentage yield of the neem seed extract using n-Hexane, ethyl acetate, methanol and aqueous solvent. From the result, higher yield of extraction (50.50%) was obtained using n-Hexane, followed by Ethyl acetate (45.00 %), Methanol (38.30%) and Aqueous (35.50%). This shows that the extract has more non-polar constituents than polar constituents.

Table 2: Phytochemical Screening of the extracts from seeds of neem

Constituents	n-Hexane	ethyl acetate	methanol	Aqueous
Alkaloids	++	+++	-	-
Saponins	-	-	-	-
Tannins	-	-	-	-
Flavonoids	-	+	+++	+++
Carbohyrdrates	-	-	-	-
Steroids	+	+	+	+
Anthraquinones	+	+++	++	-
Cardiac glycosides	+++	+	++	+++
Terpenoids	+	+	+	-

Where = absent, + = low concentration, ++ = moderate concentration, +++ = high concentration.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VII July 2023

In the present study from Table 2, preliminary phytochemical screening of nine different metabolites (alkaloids, saponins, tannin, flavonoids, carbohydrates, steroids, anthraquinone, cardiac glycosides and terpenoids) were tested in four different extracts. Experiments revealed the presence of steroids, anthraquinone, terpenoids, flavonoids, cardiac glycosides and alkaloids. Ethly acetate extracts showed a high concentration of alkaloids and anthraquinone, Methanol and aqueous extracts of neem seed oil showed high concentration of flavonoids and cardiac glycosides. n-Hexane extract showed high concentration of cardiac glycosides. This agrees with a study by Timothy et al. (2011) identifying flavonoids, sugar, terpenoids and anthraquinones in Neem Seed Oil. Saponins, tannins and carbohydrates were absent in all the extracts used which is in contrast with a research with significant amount of saponins and tannins detected. (Jeba-Malar et al., 2020).

Table 3: The comparative percentage scavenging activity (SA_{DPPH}) of the different extracts of Neem Seed Oil using DPPH radicals.

Extracts	**plants**	**6.25 %**	**12.5 %**	**25 %**	**50 %**	**100 %**
Ethyl acetate	*A.indica*	25.46± 0.15^{c}	30.49± 0.14^{c}	32.27± 0.04^{c}	32.29± 0.03^{c}	33.70± 1.10^{c}
n-Hexane	*A.indica*	24.15± 0.06^{b}	24.16± 0.10^{b}	24.45± 0.06^{b}	26.07± 0.02^{b}	32.56± 0.10^{b}
Methanolic	*A.indica*	14.55± 0.12^{b}	35.46± 0.10^{a}	44.49± 0.03^{a}	44.80± 0.05^{b}	48.73± 0.09^{b}
Aqueous	*A.indica*	32.15± 0.15^{c}	41.56± 0.06^{b}	47.19± 0.02^{b}	51.19± 0.05^{a}	51.21± 0.02^{b}

At P≤0.05 there was a significant difference in the antioxidant activity of date extracts using the DPPH substrate. Values are presented as mean ± standard error of means. Ranking was done across the plants and values with the same super script are not significant.

The comparative study of the scavenging activity of the various extracts of neem seed oil, using DPPH radicals had significant scavenging activity. There was a significant increase in the scavenging activity of neem seed oil.

Table 4: The antibacterial activity of the Neem Seed Oil extracts on gram positive and gram negative bacteria isolate.

Organisms	Extracts	**6.25 %**	**12.5 %**	**25 %**	**50 %**	**100 %**
E. coli	n-Hexane	2.17± 0.12^{b}	5.80± 0.20^{b}	8.37± 0.35^{b}	11.97± 0.6^{b}	15.63± 0.32^{b}
	Methanolic	8.00± 0.58^{a}	11.33± 0.67^{a}	15.13± 0.13^{a}	17.30± 0.44^{a}	20.20± 0.49^{a}
	Ethyl acetate	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{c}
	Aqueous	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{c}
S.aureus	n-Hexane	1.50± 0.29^{b}	3.93± 0.18^{b}	6.63± 0.47^{b}	10.60± 0.21^{b}	14.80± 0.20^{b}
	Methanolic	3.67± 0.20^{a}	6.57± 0.35^{a}	10.13± 0.47^{a}	12.83± 0.27^{a}	16.87± 0.24^{a}
	Ethyl acetate	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{c}	0.00± 0.00^{d}	0.00± 0.00^{d}
	Aqueous	1.67± 0.33^{b}	3.67± 0.36^{b}	6.00± 0.33^{b}	8.64± 0.06^{c}	11.32± 0.33^{c}
	L.S.D	1.02				
	P-value	<0.0001 ****				

At P≤0.05 there was a significant difference in the antibacterial activity of the extracts from *A.indica* on the selected bacteria isolates. Values are presented as mean ± standard error of means. Ranking was done across the oil and values with the same super script are not significant.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VII July 2023

Table 5: Minimum inhibitory concentration (MIC) and minimum bactericidal and fungicidal concentration (MBC and MFC) the Neem Seed Oil extracts.

Organism	Extracts	6.25 %	12.5 %	25 %	50 %	100 %	MIC	MBC/MFC
E.coli	n-Hexane	+	+	-	-	-	12.5	25
	Methanolic	+	-	-	-	-	6.25	12.5
S.aureus	n-Hexane	+	+	-	-	-	12.5	25
	Methanolic	+	-	-	-	-	6.25	12.5
	Aqueous	+	+	+	-	-	25	50
A.niger	n-Hexane	+	+	+	+	-	50	100

(-) represent inhibition (+) represent growth

The extract (Neem seeds oil) have broad spectrum of action as it is effective on both gram-positive and gram-negative bacteria as shown in Table 4. Below 50%, the isolates are all resistant to the extracts of *E.coli*and *S.aureus* using n-hexane and methanol extract,, this shows that the Neem seed oil is effective on both the bacterial isolates (*E.coli* and *S.aureus*). This agrees with the work of Patel et al. (2002). The antibacterial effects of Neem seed oil could be attributed to the presence of chemical substances such as cardiac glycosides and anthraquinones. The result of this research agrees with another finding which shows that the Neem seed oil extract is more effective on *E. coli* compared with that of *S.aureus* with the with the same concentration. (Jahan et al.,2007).

Table 6: The antifungal activity of the Neem seed oil (*A.indica)* extract on some selected fungal pathogens

Organisms	Extract	6.25 %	12.5 %	25 %	50 %	100 %
A.niger	n-Hexane	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	7.67 ± 0.71^{a}	12.53 ± 0.47^{a}
	Methanolic	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	Ethyl acetate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	Aqueous	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
C.albicans	n-Hexane	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Methanolic	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Ethyl acetate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Aqueous	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	L.S.D	0.62				
	P-value	<0.0001 ****				

At $P\leq 0.05$ there was a significant difference in the antifungal activity of the extracts from neem seed oil on the selected fungal pathogen. Values are presented as mean ± standard error of means. Ranking was done across the extracts and values with the same super script are not significant.

The effects of different concentration of Neem seed extract on fungal isolates of *A. niger*and *C. albicans* were studied as shown in tables 8 and 9 for Neem respectively.

The 50 % n-hexane extract of Neem seed oil caused an inhibition in growth of *A.* niger,while there was no growth inhibition in *C. albicans* (Table 6).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VII July 2023

IV. Conclusion

This medicinal plant 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 activities.

The study shows that extracts of neem seed oil possess good bioactive agents, antioxidant and antibacterial activity, and therefore they could be effectively used as a natural source of antioxidants and to be detected against gram-positive bacteria.

References

1. Akihisa A, Javed MR, Rao AQ, Husnain T. Designing and screening of universal drug from neem (Azadirachta indica) and standard drug chemicals against influenza virus nucleoprotein. BMC Complement Altern Med. 2017;16(1):519.
2. Banfi, N. A., & Perveen, K. (2003). In vitro inhibition potential of Phoenix dactylifera L. extracts on the growth of pathogenic fungi. Journal of Medicinal Plants Research, 6(6), 1083–1088.
3. Batiha, G.E. & Beshbishy, A.M. (2020). Gas chromatography-mass spectrometry analysis, phytochemical screening and anti-protozoal effects of the methanolic Viola tricolor and acetonic Laurus nobilis extracts, BMC Complementary Medicine and Therapies, 20(87). https://doi.org/10.1186/s12906-020-2848-2, Accessed: 01.11.2020.
4. Gupta, F. L. A., Lima, L. M. G., Abrante, I. A., de Araujo, J. I. F., Batista, F. L. A., Abrante, Bodiba DC, Prasad P, Srivastava A, Crampton B, Lall NS. Antibacterial Activity of Azadirachta indica, Pongamia pinnata, Psidium guajava, and Mangifera indica and their mechanism of action against Streptococcus mutans. Pharmacogn Mag. 2017;14(53):76-80.
5. Jeba-Malar, G. O., Baptistuta G. I., and Pervier, f. (2020). Phytochemical screening of Baobab oil. Internation journal of chemical sciences. 6(3), 35-43.
6. Liauw, M.Y.; F. A. Natan.; P. Widiyanti.; D. Ikasari.;N. Indraswati.; and F. E. Soetaredjo. (2008). Extraction of Neem Oil (Azadirachta Indica A. Juss) Using N-hexane and Ethanol: Studies of Oil Quality, Kinetic and Thermodynamic. ARPN Journal of Engineering and Applied Sciences, 3(3), 49-54.
7. Liyana-Pathiranan CM, Shahidi F (2005). Antioxidant activity of commercial soft and hard wheat (Triticum aestivum L) as affected by gastric pH conditions. Journal of Agriculture and Food Chemistry 53: 2433-2440.
8. Molyneux., R.J., Lee, S.T., Gardener, L.E.,& Panter K.E. (2007). Phytochemicals: The good, the bad, and the ugly? Phytochem, 68 (22-24): 2973-2985. https://doi.org/10.1016/j.phytochem.2007.09.004, Accessed: 05.05.2018.
9. Nair, Y.-J.and Chanta H. E. (2005). Cancer chemoprevention with dietary phytochemicals. Nature Reviews Cancer, 3(10), 768–780. https://doi.org/10.1038/ nrc1189
10. Nehdi, I., Omri, S., Khalil, M., & Al-Resayes, S. (2010). Characteristics and chemical composition of date palm (Phoenix canariensis) seeds and seed oil. Industrial Crops and Products, 32(3), 360–365. https://doi.org/10.1016/ jindcr op.2010.05.016
11. Noui, Y., Lombarkia, O., Bekrar, A., Chibane, H., Lekbir, A., Abdeddaim, M., 2014. Comparative study of the physicochemical characteristics and antioxidant activity of three dates varieties (Phoenix dactylifera L.) grown in Algeria. Annals. Food Sci. Technol. 15, 276–283.
12. Oladeji, Temitope Olabisi and Opaluwa, Ojotule. (2019). Phytochemical, Proximate and Antifungal Studies on Phoenix dactylifera L. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 3 Ver. IV (May – June 2017), PP 78-83 www.iosrjournals.org
13. Pandey, A. A., Yakubu, M. A. (2014). Preconditioning with Azadirachta indica ameliorates cardiorenal dysfunction through reduction in oxidative stress and extracellular signal regulated protein kinase ignaling. Journal of Ayurveda andIntegrative Medicine, 7(4), 209–217. https://doi.org/10.1016/j.jaim.2016.08.006.
14. Sahira-Banu, K. and Cathrine, L. (2015). General techniques involved in phytochemical analysis. International journal of advanced research in chemical sciences. 2 (4), 25-32.
15. Sai-Ram., & Barlow, P. J. (2000). Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry, 88(3), 411–417. https:// doi.org/10.1016/j.foodc hem.2004.02.003
16. Sofowora A (2008). Medicinal Plants and Traditional Medicine in Africa. Third edition, Spectrum Books limited, Ibadan, Nigeria. Pp 289. Thite V, Chavan R, Aparadh T & Kore A (2013). Preliminary phytochemical screening of ome medicinal plants. International Journal of Pharmaceutical, Chemical and Biological Science, 3(1): 87- 90.
17. Tesfaye, B.; T. Tefera.; O. Misikir.; and G. Tsegaye. (2018). Extraction and Comparison of Essential Oil from Neem Seed by Using Soxhlet Extraction and Simple Distillation Methods. International Journal of Engineering Technologies and Management Research, 5(9), 74-81. https://doi.org/10.29121/ijetmr.v5.i9.2018.291.