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Phytochemicals content and *in-vitro* antioxidant properties of *Azadirachta indica* seeds, leaves and twigs prepared from different extraction techniques

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Abstract

The current study was design to exploit the *Azadirachta indica* leaves, seeds and twigs for %yield, phytochemicals content and antioxidant activities using infusion, hydroalcoholic, decoction and microwave extraction techniques. The phytochemicals contents were determined by standard reported methods. While antioxidant activity was assessed by three (03) standards In-vitro antioxidant test systems as 1, 1'-diphynyl- 2-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2) scavenging activity and Nitric oxide (NO) scavenging activity method using ascorbic acid as standard. The highest extractive yield was observed in seed were 44.00±02% (infusion), 38.00±00% (Hydroalcoholic) and 20.00±03% (decoction). Followed by leaves and twigs parts of neem. The high amount (+++) phytochemicals contents were extracted from neem leaves infusion and hydroalcoholic extracts as compared to seeds and twigs neem parts with decoction and microwave extraction methods. The highest *Azadirachta indica* (neem) leaves infusion extract showed at 1000 mg/L remarkably inhibited DPPH inhibition activity (85±01), H_2O_2 scavenging activity (83±01%), and NO scavenging activity (78±01%). The antioxidants activities showed a dose dependant manner, the higher the concentration showed high antioxidant activities. The results pointed to the significant antioxidant activities of the leaves infusion and hydroalcoholic extraction techniques, the overall strength being in the order of infusion >hydroalcoholic >decoction >microwave in leaves, seeds and twigs extracts. In all cases, the extracts obtained from leaves showed higher antioxidant activity and higher phytochemicals contents than the other extraction technique obtained from seeds and twigs. The results indicate that *Azadirachta indica* leaves, seeds and twigs extracts have potent antioxidant activities that would have beneficial effects on human health and infusion extracts are superior with better antioxidant potential.

Keywords: Diseases, extraction techniques, flavonoids, free radicals, human health, natural antioxidant, neem, phenols.

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1. Introduction

Azadirachta indica is commonly known as 'neem' and the main multitalented therapeutic tree possessing a broad range of pharmacological and biological activities. Neem is found in subtropical and tropical countries like Indonesia, Thailand, Sri Lanka, Pakistan, India and Nepal. It is belongs to Meliaceae family with broad-leaved evergreen that rises up to thirty meter height. It is recognized for its ethno-medicinal and therapeutic characteristics ever since prehistoric period (Pokhrel *et al*., 2015). The United Nation announces *Azadirachta indica* the mainly significant therapeutic plant universally as the "Tree of the 21st century". Neem

has been widely utilized in Unani, homeopathic and Ayurveda medicine because its root, stems, barks and leaves has a number of therapeutic characteristics (Pokhrel *et al*., 2015).

Medicinally, neem raw extracts of leaves and bark have been exercised in traditional medication to manage illnesses like respiratory system, intestinal helminthiasis and leprosy. There are numerous additional research data on the pharmacological and biological potential like antiparalitic, antiseptic, antipyretic, anti-inflammatory, antifungal, antibacterial and antiviral uses (Britto and Sheeba 2011; Eshrat and Ali 2002; Prieto *et al*., 1999). Because of its therapeutic significance it is utilized to synthesized formulated medication for the management of diverse human diseases. Neem twigs applied by the community traditionally for shining their teeth. Neem juice drink is generally used as to destroy intestinal worms, treat fever and a good tonic to boost hunger (Pokhrel *et al*., 2015). Ameobic dysentery and diarrhea are treated with the help of stem bark. The flowers are exploited as minerals energizer, and for the healing of nasal polyposis and fever. The leaves are generally used as pesticides, minerals tonic and or the treatment of fever (Sithisarn *et al*., 2006).

The compounds are known as free radicals that do not have a colleague as a result these compounds turn into high reactive and unstable form. As the quantity constantly rises, the quantity of free radicals and antioxidant turn into disturbed, as a result oxidative cell stress produced. If this not stopped, it produced chronic ailments like cancer, diabetes, inflammation and heart attack (Haryoto and Ismi 2020). A significant therapeutic ability of herbs is their antiradical potential opposed to free radicals (Biney et al., 2020). The compounds known as antioxidants are compounds which can slow or delay the oxidation cycle, stop the oxidation through O_2 binding competitively and delay the initial stage, the propagation period blocking and stabilize or inhibit the catalyst H_2O_2. The substance naturally could remove from the body the free radicals and consequently diseases prevention occurred (Supriyanto et al., 2020). Antioxidants are of two types; synthetic and natural, both have the capacity to to inhibit oxidation processes and scavenge free radicals. Non-natural antioxidants are applied in food industries due to its efficient performance however it creates toxic and side effects on human health. Consequently, there natural antioxidant gained popularities, which are presents in therapeutic plants e.g., polyphenols (Abdullahi et al., 2020). Nowadays natural antioxidants are required in huge amount by industries. The food, cosmetic and pharmaceutical industries rely on natural antioxidants. The strong pharmacological activities of therapeutic herbs as well as to stay away from the toxic effects that originate from the use of artificial antioxidants, medicinal plants are the best choice (Abdulaziz *et al*., 2017). The phytochemicals (flavonoids, tannins and phenols) application, which have a capabilities for antioxidant qualities, to alternate numerous man-made chemicals addition and on discovering extra probable curative profits of antioxidants. So it is need of the day to carry out the research on theses phytochemicals qualitatively or quantitatively (Maisa and Claudemir 2021). So keeping in view the synthetic antioxidant limitation and neem smartness the current study was designed. Thus, the objective of this research was to investigate comparative %yield, phytochemicals and antioxidant activity of leaves, seeds and twigs of *Azadirachta indica* from extraction techniques including infusion, decoction, hydroalcoholic and microwave.

2. Materials and Methods

The leaves, seeds and twigs of *Azadirachta indica* were collected from Board Bazar, Jamrud Road Peshawar, Khyber Pakhtunkhwa-Pakistan. The collected plant materials were dried in an Air Cabinet Dryer (England) at 35 °C for 3 days. The dried leaves were grinded in a Waring® Commercial Laboratory Blender USA.

2.1. Extraction methods

2.1.1. Infusions

The infusion of *Azadirachta indica* leaves, seeds and twigs were carried out by described infusion method of Natalia *et al*., 2015 with the minor alterations. Infusions were synthesized by the addition of boiling distilled water 200 mL to five gram (05g) sample. Then, they were stay put to stand at room temperature and kept covering overnight with piece of aluminum foil. Lastly, the blended material was filtered through Whatman®40 filter paper 125mm Ø Whatman International Ltd. Maidstone- England for five minutes and after that filtered under low pressure.

2.1.2. Decoction

The leaves powder of *Azadirachta indica* was extracted through boiling with deionized H_2O water (1:20, w/v) for six hours and subsequently filtered. Additional segment of the deionized H_2O were supplemented to the residue and the extraction was repeated until the final filtered portion obtained colorless. The collective extract was filtered through Whatman®40 filter paper 125mm Ø Whatman International Ltd. Maidstone- England. The evaporation of the filtrate was carried out on a boiling water bath until steady mass was achieved to give the extract called decoction. This process was repeated for *Azadirachta indica* seed and twigs powder (Worarat and Wandee 2009).

2.1.3. Hydroalcoholic Extractions

The hydroalcoholic extraction of *Azadirachta indica* leaves, seeds and twigs were practiced by extraction technique designed by (Natalia *et al*., 2015) among little amendments. Hydroalcoholic extractions were carried out by mixing the five gram (05g) plant material with 100 mL of methanol/water (80:20, v/v) for one hour at 150 rpm and 25 °C and filtered through Whatman®40 filter paper 125mm Ø Whatman International Ltd. Maidstone- England. The remains deposit was subsequently pull out with 01 extra thirty milliliter part of the mixture of hydroalcoholic. The collective extracts were evaporated under low pressure (Rotary

Evaporator Büchi R-200, Switzerland; B-490 Heating Bath- Buchi) at 35 °C and then further dried on water bath with a constant weight.

2.1.4. Microwave assisted extraction (MA)

Microwave oven (Panasonic) a commercial household was applied for the extraction of microwave technique. The condition of microwave oven for operation was single-phase output of 800 W at 2450 Hz. Ten gram (10.0g) portion of the sample was kept in a flask of six hundred milliliter (600ml) capacity, followed by mixing of two hundred milliliter (200ml) of deionized H_2O. The cycle of irradiation conditions was as: Three minutes for pre heating (70°C), power-ON for one minutes, followed power-OFF for two minutes to grip hotness (70-85°C), the process of extraction was after that recurred eight cycle. The extraction process subsequent to finishing, at room temperature the flask was keep for cooling. Then filtration of the mixture was carried out with the help of Whatman®40 filter paper 125mm Ø Whatman International Ltd. Maidstone- England. The solvent additional parts were mixed to the experimented residue and the extraction was recurring until the end extract showed no color. The extract obtained was subsequently kept on boiling water bath for evaporation until to achieve stable weight, which was called microwave extract (Worarat and Wandee 2009).

2.2. Qualitative Phytochemicals Analysis

The basic phytochemicals qualitative determinations were examined by the following authentic standards procedures (Khanal 2021).

2.2.1. Saponins: Test of Foam: The solution of neem extract was diluted with deionized H_2O and keep in a test tube. For a few minutes suspension was formed. A layer of foam 2cm gives a positive sign of saponins.

2.2.2. Alkaloids: To test alkaloids in a neem extracts samples the Mayer's reagent was applied. In a test tubes two milliliter of neem extract was taken and added on it two-three drops of Mayer's reagent. The emergence of green coloration precipitate in the solution was a sign of alkaloid. Wagner's reagent was used to carry out Wagner's test. Take 2ml of neem extracts in test tube and added a few drops of Wagner's reagent. As a result precipitate of brick color appearance showed the occurrence of alkaloids.

2.2.3. Flavonoids: To detect the flavonoids in neem extracts alkaline reagent test was performed. Take two milliliter (2ml) of neem extracts in test tube and solution of NaOH (2% w/v) 2ml was also mixed with it. In the test tube a strong yellow color was appeared. Then dilute HCl few drop was added. The mixture was colorless, which denoted the occurrence of flavonoids.

2.2.4. Phenols and Tannins: The neem extract was blend with two milliliter of $FeCl_3$ solution (2%). The appearance of blue-black or blue green color signified the existence of tannins and phenols.

2.2.5. Terpenoids: Neem extract was blended in two milliliter (2ml) of chloroform and was evaporated till to dryness. Concentrated sulphuric acid 2ml was added; at the interface a reddish-brown color give a positive sign for of terpenoids.

2.3. In Vitro Antioxidant Activities

2.3.1. Scavenging procedure of DPPH

Different concentrations of neem extract/standard (200, 400, 600, 800 and 1000 mg/L) were prepared in 95% (methanol) and blend this 1mL extract solution with 1 mL 95% (methanol) solution of 0.004% DPPH and also with standard (vitamin C) solution individually. As a blank DPPH and methanol (95%) with 1:1 mL was prepared. These experimented materials were reserved for twenty minutes at dark room. Then the absorbance was calculated on U-2900, Hitachi - UV/VIS Spectrophotometer (Tokyo Japan) at 517nm. The following equation was used to calculate the scavenging inhibition % (Jong *et al*., 2010).

$$\text{Scavenging Activity \%} = \frac{(A0 - A1)}{A0} \times 100 \quad (1)$$

where A0 = Control Absorbance, A1= Sample Absorbance

2.3.2. Hydrogen peroxide scavenging activity

An aliquot 2 mM of H_2O_2 and neem extract/standard (200, 400, 600, 800 and 1000 mg/L) concentrations were blended (1:0.6 v/v) and for ten minutes at room temperature incubation was carried out. Then, absorbance reading was noted by using U-2900 Hitachi-UV/VIS Spectrophotometer (Tokyo-Japan) at 230 nm against a control solution having phosphate buffer lacking H_2O_2. The H_2O_2 scavenging activity (%) was measured from equation 1 (Arvind *et al*., 2013).

2.3.3. Nitric oxide scavenging activity

The neem extract/standard (200, 400, 600, 800 and 1000 mg/L) 01mL was blended with 1 mL of the fusion solution holding 10 mmol/L (sodium nitroprusside) in phosphate buffer (pH 7.420; mmol/L). The reaction solution was incubated for one hour at 37 °C, and 0.5 mL of Griess reagent was then blended with 0.5 mL of the aliquot. The absorbance was calculated by U-2900 Hitachi-UV/VIS Spectrophotometer (Tokyo, Japan) at 540nm. The scavenging activity of nitric oxide was analyzed using the formula given in equation 1 (Otakar *et al*., 2014).

3. Statistics

Every testing trail was carried out in triplicate and findings are given as an averages ± SD. $p<0.05$ value was assumed as significant.

4. Results and Discussion

Table 1 shows the extractive yield (%) values of *Azadirachta indica* parts. The results showed that the maximum and minimum extractive values were 44.00±02 % and 05.00±01% calculated by seeds from infusion and twigs from decoction. It was observed that seeds were the highest extractive values, leaves were moderate and twigs were the least. The extraction methods extractive values were increased in the order infusion> hydroalcoholic> decoction> microwave.

Table 1. Extractive Yield (%) of Azadirachta *indica* Parts.

Extracts	Solvents	Leaves	Seeds	Twigs
Hydroalcoholic	Aqueous and Alcohol	12.00±00	38.00±00	07.00±00
Infusion	Aqueous	13.00±02	44.00±02	08.00±01
Decoction	Aqueous	19.00±03	20.00±03	05.00±01
Microwave	Aqueous	08.00±01	09.00±01	06.60±01

Each value represents an average of three replicate ±SD.

4.1. Phytochemical Composition

The therapeutic values of herbs are because of the occurrence of significant chemical molecules that have define biological effects on the living organism. Tannins, flavonoids, phenols, steroids, saponins, glycosides and alkaloids are the most important phytochemicals. Phytochemical screening of neem leaves, seeds and twigs showed the presence of alkaloids, phenols, flavonoids, saponins, tannins and terpenoids. Table 2 shows the results of the qualitative analysis of the metabolites investigated in this work. Alkaloids are nitrogenous organic molecules. Their behavior is alkaline and displays pharmacological activities with an extra ordinary manner. Certain alkaloids work as respiratory and cardiac stimulants. They are also applied for numerous kinds of cancer treatment (Garima *et al*., 2014). In the current study the infusion and hydroalcoholic extraction techniques showed the large quantity (+++) of alkaloids in the neem leaves. Neem seeds observed average quantity (++) of alkaloids for infusion and hydrolacoholic extraction techniques. The small quantity (+) of alkaloids was noted in decoction and microwave extraction methods. Twigs showed small quantity (+) of the alkaloids in all extraction methods.

Table 2. Phytochemical Classes of *Azadirachta indica*

Extracts	Parts	Extraction Techniques			
		Infusion	Decoction	Hydroalcoholic	Microwave
Alkaloids	Leaves	+++	++	+++	++
	Seeds	++	+	++	+
	Twigs	+	+	+	+
Phenols	Leaves	+++	++	+++	++
	Seeds	++	++	++	+
	Twigs	+	+	+	+
Flavonoids	Leaves	+++	++	+++	+
	Seeds	++	++	++	+
	Twigs	+	+	+	+
Saponins	Leaves	+++	+++	+++	++
	Seeds	+++	++	++	+
	Twigs	+	+	+	+
Tannins	Leaves	+++	++	+++	++
	Seeds	++	++	++	++
	Twigs	+	+	+	+
Terpenoids	Leaves	++	++	+++	++
	Seeds	+	++	++	++
	Twigs	-	+	-	+

+ sign shows detection level of the phytochemicals present in extracts. + = small quantity, + + = average quantity, + + + = large quantity, _- = not detected

Flavonoids are extensively disseminated in higher plants. Flavonoids work as antioxidants which give defense in oppose to free radicals that harm tissues and cells. The phytochemicals such as saponins, flavonoids, glycosides and alkaloids are providing defence system to plants. These defence mechanisms are really the antibiotic principles of the herbs against their diverse enemies (Hafiza 2000). The present study observed that phenols and flavonoids are found in large quantity (+++) in infusion and hydroalcoholic extraction system of neem leaves. Similarly neem seeds shows average quantity (++) of phenols and flavonoids in decoction, infusion and hydroalcoholic extraction techniques. The microwave extraction method shows a small quantity (+) of phenols and flavonoids in seeds and twigs parts of neem.

Saponins were reported anti-protozoal effects, antibacterial and antifungal properties (Garima *et al*., 2014). Our current study report that saponins found in large quantity (+++) in hydroalcoholic, decoction and infusion extraction system in neem leaves; seeds infusion extraction technique found saponins in large quantity (+++). Twigs share a small quantity of saponins in all extraction system.

Tannins are effectual in peptic ulcer, colitis, diarrhea and promote wound healing (Hafiza 2000). The present study observed that tannins are present in large quantity (+++) in neem leaves infusion and hydroalcoholic extract; Seeds found average quantity (++) and twigs investigate small quantity (+) of tannins for all extraction protocols.

A high amount of polyphenolic and phenolic substances are present in *Azadirachta indica*, but depend on the environmental factors and geographical location (Kaushik *et al*., 2007). The absence or presence of define categories of phytochemicals occurs in the parts of *Azadirachta indica* might be differ according to climatic aspects and geographical position. These statements are shared by (Shuaibu *et al*., 2015 and Ghimeray *et al*., 2009) in their research on neem phenolic derivatives quantification. Moderate amounts of flavonoids, tannins and phenols were found in neem alcoholic extracts in semi-arid regions of Nigeria (Benisheikh *et al*., 2019). But in our present study the hydroalcoholic extract shows large quantity (+++) of studies phytochemicals in neem leaves, average quantity (++) for seeds and small quantity (+) in neem twigs (except terpenoids absent in twigs hydroalcoholic extract). The differences in phytochemicals contents is due to extraction techniques, solvent system, plants parts, geographical variation, powder mesh size and other operation condition (e.g. temperature, time etc.).

Terpenoids present in large quantity (+++) in hydro-alcoholic extract of neem leaves; average quantity (++) are found in infusion (leaves), decoction (leaves and seeds) and microwave (leaves and seeds); small quantity (+) are found in seeds (infusion), twigs (decoction and microwave). Terpenoids are not detected (-) in twigs infusion and hydroalcoholic extracts.

Contrast with the additional techniques, decoction technique was convenient, simple and low-priced. The decoction extraction method due to its feasibility can be applied in other nation state, particularly in budding countries (Sithisarn *et al*., 2006). However, many new extraction techniques have been developed and studied for plant extracts preparation; these comprise pressurized liquid extract (Ong *et al*., 2003), microwave assisted extraction (Wang *et al*., 2004) and ultrasonic extraction (Soares *et al*., 2006). These techniques have diverse benefits that comprise short extraction temperature contact which affects the steadiness of vigorous ingredients and enhancement of the diffusion of solvent into herbs powder for ultrasonic extraction (Soares *et al*., 2006). The techniques such as microwave-assisted endorse improved production and quick the process of extraction for targeted bioactive molecules like glycyrrhizic acid in liquorice (Wang *et al*., 2004). The polar and thermal labile bioactive ingredients like glycyrrhizin, baicalin and berberine could be extracted using pressurized liquid extraction method (Ong *et al*., 2003). Therefore, these techniques for herbal extraction may perhaps endorse the effectiveness of good quality extraction of precise bioactive substances and decrease period of extraction. However, these extraction techniques have costly as compared to the decoction techniques. So far, these up to date techniques must be measured for extracting higher quantity and quality of *Azadirachta indica* leaves for medicinal pharmaceutical manufacture (Sithisarn *et al*., 2006).

4.2. Antioxidant Activities

The DPPH radical scavenging activity of neem is shown in Figure 1. The extent of DPPH radical scavenging at different concentrations (200-1000 mg/L) was measured, with ascorbic acid as the standard. The inhibition activity of the plant extract was comparatively lower than the ascorbic acid. The DPPH scavenging activity of neem shows in the figure data that free radical inhibition % increased as the concentration increased in all extraction systems. However, the radical inhibition % were higher (85±01 @1000mg/L) in infusion extract of leaves when compared with other extraction system against seeds and twigs of neem. The DPPH scavenging activities observed that leaves showed the highest activities; seeds showed the moderate activities and twigs calculated the least DPPH scavenging potential. The extraction system comparison revealed that infusion is the most efficient, followed by nydroalcoholic, decoction and microwave extraction methods. The standard (vitamin C) DPPH scavenging activity was high 90±0.8% at 1000mg/L.

The H_2O_2 scavenging activity of Neem is shown in Figure 2. According to these results, there were significant differences between antioxidant activity between plants parts, extraction techniques and extracts dose. These data observed that H_2O_2 scavenging activity showed a dose dependent manner, more the concentration high the scavenging activities. The highest H_2O_2 scavenging activity (83±01% 1000mg/L) is observed in neem leaves agents infusion extraction methods. While the lowest H_2O_2 scavenging potential (48±0.5% @1000mg/L) is calculated in neem twigs against microwave extraction technique. The positive control (Vitamin C) found 86±01% H_2O_2 scavenging activity at 1000mg/L concentration.

As shown in the Figure 3, the nitrite oxide radical scavenging activity of neem leaves, seeds and twigs using infusion, hydroalcoholic, decoction and microwave extraction system. All studies neem parts extracts nitric oxide scavenging activities

increased as the concentration raised (200 mg/L to 1000 mg/L). The maximum nitric oxide scavenging activity was achieved by neem leaves extract (infusion) 78±01% at the concentration of 1000mg/L. The neem parts nitric oxide scavenging activities were increased in the order leaves>seeds>twigs. The extraction techniques nitric oxide scavenging activities were increased in the order infusion>hydroalcoholic>decoction>microwave.

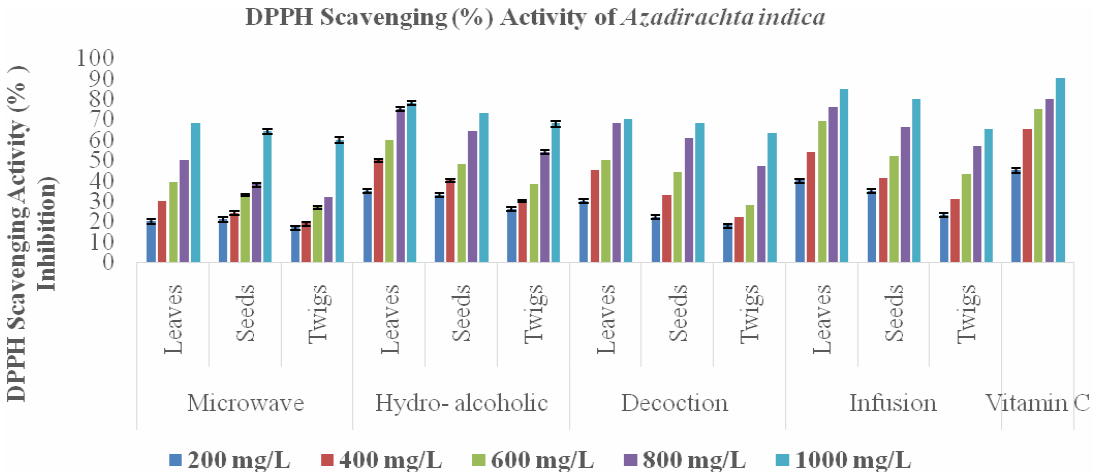

Fig.1. DPPH Scavenging (%) Activity of *Azadirachta indica*.

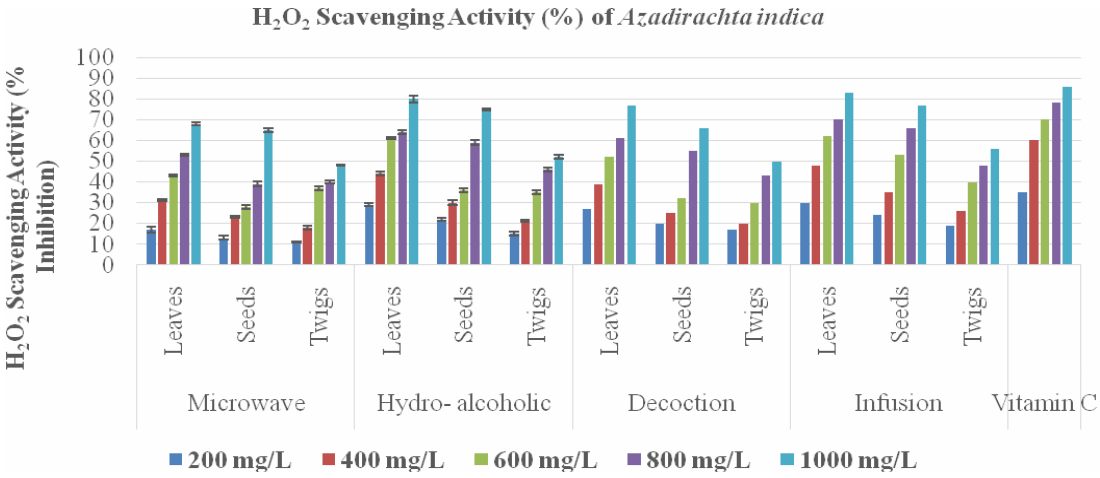

Fig.2. H_2O_2 Scavenging Activity (%) of *Azadirachta indica*.

A lot attention has been given to natural antioxidants in recent years because of their healthiness profit. Drug formulations based on antioxidant are applied for the treatment and prevention of numerous multifarious ailments. Herbs are the main foundation of natural antioxidants; they synthesized a broad spectrum of chemical compounds with radical scavenging potential that have therapeutic potential. The most plentiful antioxidant molecules of herbs crude substance are Polyphenols.

It is recognized that numerous diseased such as AIDS, cancer and neurodegenerative diseases associated with free radicals. Due to the scavenging potential of the antioxidants, they are helpful to supervise these diseases (Sylvie *et al*., 2014). Measurement of antioxidant activity in the plants extracts samples is used to determine the absorbance of stable radicals DPPH spectrophotometrically. This technique is sensitive, reliable and most rapid (Waghulde *et al*., 2011).

DPPH can accept hydrogen radical or electron to form an unwavering diamagnetic particle. Generally it is a free radical. All the neem extracts investigated scavenge the DPPH radical however, in diverse behavior. These finding showed that the neem extracts are the ability to donate hydrogen or electron which may fuse with DPPH radical. The differences noted between the reducing potential of the same extract depend on the piece of herb tested in the study. It was reported that variation of antioxidant activity might be due to an irregular division of the antioxidant substances like flavonoids, polyphenol detected in the diverse piece of plant (Sylvie *et al*., 2014). Extracts are composed of a combination of numerous antiradical molecules which may perhaps work in

a synergetic mode to improve the scavenging potential. Furthermore, the extracts scavenging activities to grip trap radical depends on the ability and availability of these extracts to provide electron or hydrogen atom (Devi *et al*., 2008).

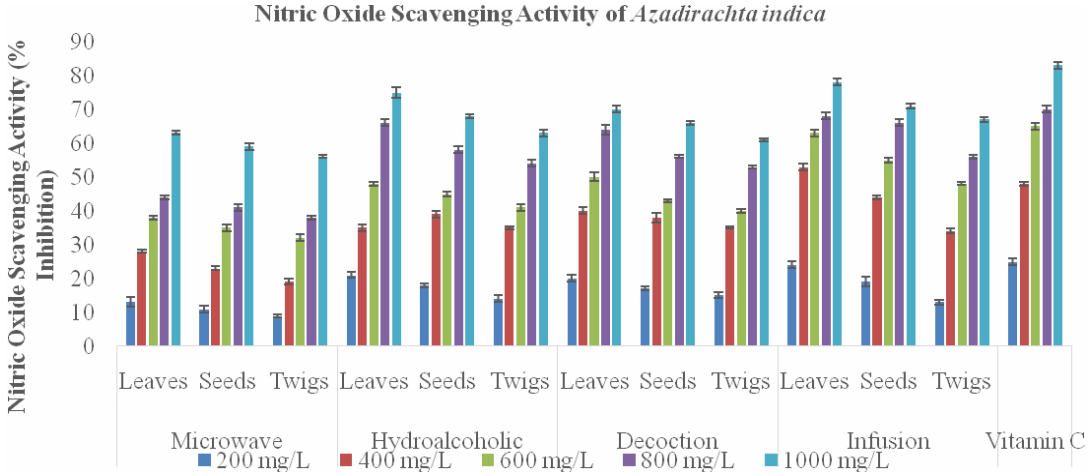

Fig.3. Nitric Oxide Scavenging Activity of *Azadirachta indica*.

The role of nitric oxide in numerous inflammatory systems is well recognized. Continuous synthesis levels of nitric oxide are proportionally lethal to cells and lead to fall down of vascular system (Hazra *et al*., 2008). All the neem extracts illustrated that it have the capacity to inhibit scavenge and nitrite radical. Our results data demonstrate that these extracts might work either scavenger or free radical inhibitors applying their actions as the primary antioxidants or could act as proton-donator. It was reported that the extract stop the nitrite radical synthesis by straightly contending with O in the fusion which might show the way to the nitrite synthesis inhibition (Yin *et al*., 2007). The non inhibition or the accumulation of the radical (nitrite) synthesis in the organisms tissues might be poisonous and encourage mutagenic fusions with superoxide radical, synthesizing the extremely active peroxynitrite anion (Sylvie *et al*., 2014). The current study provide evidence that the neem leaves infusion extracts have powerful nitric oxide inhibition abilities and might be helpful to manage nitrite radical engage illness.

Secondary metabolites such as phenolic compounds are accountable for the antiradical activity demonstrated by numerous natural products sources. This relationship was well proof in a research conducted in China on one hundred and twelve (112) customary herbs; neem leaves along with among (Cai *et al*., 2004). Antioxidant activities of neem leaves from India (Wandscheer *et al*., 2004), England (Salehzadeh *et al*., 2003), Nepal (Ghimeray *et al*., 2009), Brasil (Nagano and Batalini 2021), Thailand (Sithisarn *et al*., 2006), Nigeria (Olaitan *et al*., 2021) have also been reported. Neem leaves and oil were reported maximum antioxidant activity % because of its phenolic compounds and enormous scavenger ability towards free radical (Nikolova et al., 2022; Nakamura et al., 2022). These observations are a close agreement to the current study.

Our results confirmed that the infusion extraction was relatively high quantity of secondary metabolites as compared to decoction. Different phytochemicals are less in decoction than infusion explaining that infusion is a better and enhanced extractive technique as compared to boiling in H_2O. Parallel results was documented in previous research by (Fotakis *et al*., 2016) who concluded that rising boiling period in decoction technique decrease concentration of phytochemicals as well as their pharmacological activities. It was reported that decoction method gave a low yield but high antioxidant activity, indicating that the active antioxidant components in the *Azadirachta indica* leaves were better extracted by hot water as compared to maceration (ethanol) method (Sithisarn *et al*., 2006). Dissimilar findings could be due to the maintenance process, harvesting process, plant age, concentration taken and others (Haryoto and Ismi 2020).

The research data disclosed that leaves and bark extracts of neem displayed antiradicals' activities and acquire phenolic compounds in a dose dependent behavior. On the other hand, the extract of neem bark demonstrated the highest actions as compared to the extract of leaves (Olaitan *et al*., 2021). But in our research the neem leaves as well as seeds extracts have a higher antioxidant activities as well as higher phytochemicals contents. These variations are explained by the differences in neem geographical condition, extraction methods, plants maturity stages, plants parts, solvents system and other experimental conditions variations.

In current era, the exploration for secondary metabolites having antiradical characteristics has been augmented because of their abilities utilization in the treatment in numerous infectious and chronic illnesses. The neem extracts phytochemical screening revealed the presence of polyphenols, flavonoids, glycosides, alkaloids, tannins and saponins. The antioxidant activities acquired were in great share because of the occurrence of phenolic groups in the chemical composition of the secondary metabolites, mostly in tannins and phenols, which are noticeably occurred in the infusion, hydroalcoholic, decoction and microwave extracts of neem leaves and seeds, as wells as in little quantity in twigs.

5. Conclusion

On the basis of the present study, it could be concluded that leaves and seeds of *Azadirachta indica* extract pronounced polyphenol concentration and show high antioxidant properties. Their curative power against a range of diseases might be exploited, especially those associated with oxidative stress. The experimental data reveal that all the leaves extracts are likely to have the effect of scavenging free radicals and thus can be incorporated into food, medicine as well as in cosmetics for healthy skin and/or anti-ageing products. However, extensive investigations need to be done either to isolate the antioxidant compounds or to determine in-vivo biological and pharmacological activities of these extracts. Further research will be carried out regarding formulation of food and nutraceuticals products from neem, which should be a new option to manufacture secure antioxidants related food/medicine/cosmetic products. Indeed, some pilot scale and industrial production studies will be designed to establish the neem based industrial setup in poor and developing countries to create employment opportunities and reduce the imports of food, medicine and cosmetics products within the neem based products domain.

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