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DETERMINATION OF THE ANTIOXIDANT CAPACITY OF DIFFERENT SOLVENT EXTRACTIONS OF LEAVES AND BARK OF AZADIRACHTA INDICA (NEEM)

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ABSTRACT

Antioxidants diminish the activation of overwhelming free radicals which is the cause for many diseases. As using synthetic antioxidants carries drawbacks, at present there is a high demand on natural antioxidants derived from medicinal plants to develop natural therapeutic strategies. The present study aims to detect the antioxidant properties of the leaves and bark of the versatile medicinal plant, Azadirachta indica (Neem). The dried and powdered leaves and bark samples were extracted via cold maceration with four differently polar solvents:80% ethanol, 80% methanol, distilled water and chloroform. A qualitative phytochemical screening was performed to detect various phytochemicals resulting a higher number of phytochemicals in Neem leaves. The quantitative determination of the total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) was conducted using spectrophotometry with reference to Gallic acid, Rutin and Ascorbic acid standard curves respectively. Furthermore, the free radical scavenging activity was obtained in terms of 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS), Ferric ion reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical activity. The statistical analysis was done via SPSS. The study revealed that extracting a particular agent depends on the solvent and the extracted part of the plant. With reference to that, polar solvents showed more contribution towards the extraction while leaf extracts stated a higher antioxidant capacity, established by the TPC values of ethanolic and chloroform leaf extracts which were 508.58 µgGAE/g and 181.65 µgGAE/g respectively. The present study can be developed further to investigate alternative drug developments using the antioxidant properties of Neem.

Keywords: Antioxidant capacity, Azadirachta indica, free radicals, solvent extractions

INTRODUCTION

Background

To ensure the survival and well-being of the body, humans indulge activities such as inducing the synthesis or release of reactive substances (Engwa,2018) that are generated due to various endogenous and aerobic metabolic reactions in the human body or by exposure to various physiochemical statuses (Lobo et al.,2010). These reactive species are nitrogenated/oxygenated free radicals containing an unpaired electron in their outermost orbital and are capable of independent existence. When in a balanced state, they functionate in regulatory processes such as cell proliferation, apoptosis and gene expression (Pisoschi et al.,2016). When

produced in excess, free radicals counteract on macromolecules resulting in cell damage and oxidative stress that arises when balance between free radicals and antioxidants are unfavorable. Oxidative stress leads to chronic diseases like diabetes, atherosclerosis, rheumatoid arthritis and cancer (Liguori et al.,2018).

Antioxidants are categorized as substances that inhibits oxidation. They are capable of terminating the damage caused by free radicals and oxidative stress by their free radical scavenging ability. Antioxidants can be synthesized in-vivo or consumed in the diet. Plants are considered as the main source of exogenous antioxidants, because of their abundancy in phytochemicals, that possesses antioxidant properties, (Kasote et al.,2015). These natural antioxidants are investigated in therapeutic interventions to cure many diseases due to its less toxicity and minor harmful effects.

The present research aims on determining the best solvent for the extraction of neem leaves and bark out of four different solvents, to observe their antioxidant capacities and free radical scavenging properties. It focuses on the determination of the antioxidant capacity of the plant materials with reference to the TPC, TFC and TAC and determination of the free radical scavenging activity using DPPH, FRAP and ABTS assays.

Introduction to the plant Neem

The versatile herbal plant Azadirachta indica, commonly known as Neem, is a flowering plant that has a life span of 150-300 years and grows approximately 15-20 meters tall (Yadav et al., 2016). Neem bark is brown and rough while pinnate Neem leaves grow up to 30 centimeters long with 10-12 serrated sets of leaves. (Maragathavalli et al.,2012). Neem is a member of Meliaceae family and is mainly cultivated in the Indian subcontinent. As recorded Neem was initially used in the ancient Indian Harappa culture for medical treatments and currently almost every part of the tree is extensively used in the fields of Ayurveda, Unani and Homoeopathic medicines to treat various ailments as listed in the table 1.0 (Kumar and Navaratnam,2013).

Table 1.0 Medicinal uses of different parts of Neem (Adapted from Agrawal,2002 and Sahrawat et al., 2018)

Part	Medicinal Use
Leaf	Anorexia, biliousness eczema, epistaxis, eye problem, leprosy, intestinal worms, ringworm acne, skin ulcers, smallpox chicken pox, malaria
Bark	Analgesic, alternative and curative of fever
Flower	Bile suppression, elimination of intestinal worms and phlegm
Fruit	Piles. Intestinal worms, urinary disorder, epistaxis, phlegm, eye problem, diabetes, wounds and leprosy
Twig	Cough, asthma, piles, phantom tumour, intestinal worms, spermatorrhoea, obstinate urinary disorder, diabetes
Gum	Scabies, skin diseases, wounds, ulcers,
Seed	Intestinal worms and leprosy

Neem tree as a whole is an abundant source of antioxidants and performs a significant role in scavenging free radicals, that impact the growth and development of certain definite conditions of the tree. Active components of Neem leaves, bark, seeds and oil which is extracted from Neem seeds are considered as strong anti-inflammatory agents and consists of antimicrobial, anticancer, anti-arthritic properties. An active agent of Neem recognised as NLGP is known as a potent immunomodulatory agent. (Wilson,2019 and Chaudhary et al.,2017). Moreover, Neem contains hepato-renal protective activity and hypolipidemic effects (Sahrawat et al., 2018). The antibacterial properties of the plant could be used to control air bone bacterial contaminations (Maragathavalli et al.,2012). Such important therapeutic properties of Neem can be effectively used in synthesizing new phytomedicines (Fong et al.,2014). Therefore, as Neem is recognised as an anti-cancer agent, currently the ethanolic extracts from neem leaves are tested to promote treatments to

hinder mammary tumour activity (Braga et al.,2018).

Apart from the medicinal benefits, the insecticidal properties of the leaves, bark and oil which can be effective as pesticides, herbicides, fungicides and weedicides in agriculture (Chaudhary et al., 2017). Figure 1.0 illustrates a summary of the bio pesticidal and therapeutic properties contained in different parts of Neem.

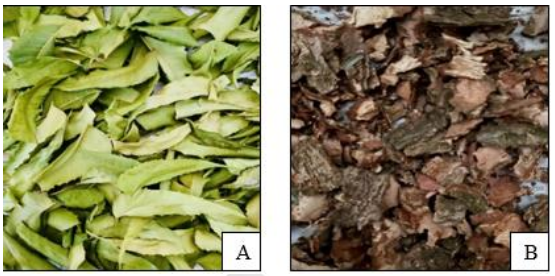
Figure 1.0 Schematic representation of the Bio pesticidal and therapeutic properties of Neem (Chaudhary et al.,2017).

Determination of phytochemicals of different solvent extractions of Neem leaves and bark.

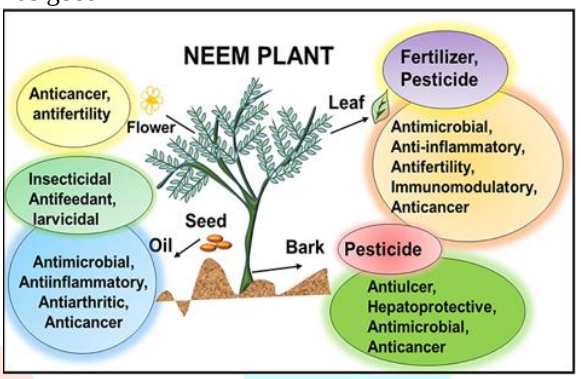
Preliminary screening of phytochemicals is the important qualitative analysis in detecting various bio-active compounds present in plants which lead the pathway for drug discovery (Yadav et al.,2014). Successful determination of any biological property mainly depends on the extraction method and the type of solvent used (Yusuf et al.,2014). In the present research, the crude extracts of dried neem leaves and bark as shown in Figure 2.0 were obtained using cold maceration technique with reference to three polar solvents: 80% ethanol, 80% methanol, distilled water and one non-polar solvent: Chloroform. The dielectric constants of these solvents are 33.0, 25.3, 80.4 and 4.81 respectively.

Figure 2.0 Dried neem samples A) leaves and B) bark

Most of the phytochemicals, are polar groups and dissolves well in ethanol and methanol, but alcohols



will be evaporated to avoid any possible false positive activities (Do et al.,2014). Ethanol emerges as a conventional solvent due to safety for infused edibles and is considered as an ideal solvent in extracting terpenes and phenolic compounds (Sun et al.,2015). Methanol is frequently used as it has good



penetration to the cell content and has efficacy in extracting lower molecular weight polyphenols (Truong et al.,2019). Phytochemicals like glycosides has a higher polarity and is sparingly soluble in these solvents therefore to compare with them aqueous extracts were used (Nawaz et al.,2020). Chloroform is used as it is relatively unreactive, conveniently volatile and miscible with most of organic compounds that are non-polar (Dhawan and Gupta,2017).

Determination of the Antioxidant capacity

The antioxidant capacity of a plant is a quantitative analysis estimated spectrophotometrically, and determined by the concentration of antioxidants. It is

an important parameter in terms of quality control and authentication of plant products (Tütem et al.,2014). Phenolic compounds (flavonoids, phenolic acid and tannins) are recognized to be the major contributors towards the antioxidant activity in plants (Sonboli et al.,2010). In this research, antioxidant activity of extracted samples was quantified by three tests: Total phenolic content (TPC), Total flavonoid content (TFC) and the Total antioxidant capacity (TAC) with reference to Gallic acid, Rutin and Ascorbic acid standard curves respectively. The following Table 2.0 indicates a description on the principle of each assay

Table 2.0 Methods and the basic principle of TPC, TFC and TAC (Adapted from Maragathavali et al., 2012)

Assay	Method	Principle
TPC	Folin-Ciocalteau micro method	Polyphenols in plant extracts reacts with Folin-Ciocalteau redox regent forming a blue complex
TFC	$AlCl_3$ colorimetric method.	$AlCl_3$ forms acid stable complexes with the keto group of 4th Carbon and either 3rd or 5th Carbon hydroxyl groups of flavones and flavanols
TAC	Phosphomolybdenum method	Reduction of Mo (VI) to Mo (V) occurs by plant extracts containing antioxidant compounds

Determination of the free radical scavenging activity

Many of the natural antioxidants are identified as potent free radical scavengers that could prevent the damage caused by excess free radicals (Sonboli et al.,2010). In this study DPPH, ABTS and FRAP free radical scavenging activities of Neem extracts were estimated spectrophotometrically. DPPH and ABTS assays are discoloration assays, in which the purple DPPH free radical reduces to a yellowish hydrazine due to the reaction with hydrogen donors (Kiranmai et al.,2011) and ABTS·+ radical was produced by the reaction between aqueous ABTS and potassium persulfate. FRAP assay is based on the reduction of colourless Fe3+-TPTZ complex to a blue colour Fe2+-tripyridyl-triazine formed by electron donor antioxidants at low pH levels (Rajurkar and Hande,2011).

Methodology

Sample preparation, Extraction and filtration

Neem leaves and bark samples were collected from a home garden located in Dehiwala, Sri Lanka, and were washed and shade dried for two weeks. Dried samples were grounded into fine powder and 8.0g of each powder was extracted with 40.0ml of each solvent;80% ethanol, 80% methanol, chloroform and water to a ratio of 1:5 and kept in the roller-mixer for 24 hours followed by a double filtration. The filtrates were transferred into clean falcon tubes and stored at -4°C until further use (Dharajiya et al.,2017).

Phytochemical screening

The filtrates were diluted to a ratio of 1:10 with their respective solvent and were tested to detect the presence of following phytochemicals as listed in Table 3.0.

Table 3.0 Phytochemical screening tests adopted from Ali et al., 2018, Bandiola, 2018, Dhruti et al., 2016 and Maragathavalli et al., 2012).

Phytochemicals	Procedure
Phenols	Distilled water (2ml) followed by 5 drops of 10% ferric chloride was added to 1ml of the extracts. Formation of blue/green colour was observed
Flavonoids	Few drops (1-5) of concentrated HCl were added to 1ml of extract. Formation of a yellow colour was observed.
Saponins	Distilled water (2.00ml) was added to 2.00ml of plant extract and shaken for 15 minutes lengthwise. A foam formation of about 1cm was observed.
Cardiac Glycosides	To each plant extract, 3.00ml of both chloroform and 10% ammonia solution was added and a formation of pink colour was observed.
Steroids	To 2.50ml of plant extract, 1.00ml of chloroform and concentrated H_2SO_4 was added and a red coloration as observed.
Quinones	To 1ml of each extract 1.00ml of concentrated sulphuric acid was added and a formation of red colour was observed.
Tannins	Ferric chloride (5%; 2.00ml) was added to 1.00ml of each of the extracts. A greenish black coloration was observed
Terpenoids *(Salkowski's test)*	To 2.50ml of each extract was carefully mixed with 1ml chloroform and 1.50ml of sulphuric acid. Reddish brown precipitate was observed.
Coumarins	To 1.00ml of the extract 1.00ml of concentrated sulphuric acid was added and a yellow colour represented coumarins
Carbohydrates *(Molish's test)*	To 2.00ml of the extract, two drops of alcoholic solution of α-naphthol were added. The mixture was shaken well and few drops of concentrated sulphuric acid was added slowly along the test-tube wall. A violet ring was observed
Fats *(Spot test)*	A small quantity of extract was dropped into a filter paper and circular oil stans were observed.
Phytosterols	To 2.00ml of the extract 2.00ml of both chloroform and concentrated sulphuric acid was added. Brown or red ring on the sulphuric layer was observed.
Phlobotannins	To 1.00ml of plant extract, few drops of 10% ammonia solution was added. A pink precipitate was observed.

Determination of the total antioxidant capacity

Determination of the Total Phenolic Content (TPC)

The Folin-ciocalteau micro method was followed according to a modified protocol adopted from Wangcharoen and Morasuk, 2007. To 60.0µl of each plant extract diluted with 4.80ml of distilled water, 300.0µl of the Folin-ciocalteau reagent was transferred. The reaction mixture was incubated for 8 minutes and was mixed with 900.0µl of 20% Na2CO3. The samples were inverted for several times and incubated for 30 minutes at 40°C. The absorbance was measured in triplicates at 765nm and distilled water was used as the blank. TPC was calculated in µg Gallic acid equivalent/g (µgGAE/g) with reference to Gallic acid standard curve.

Determination of the Total Flavonoid Content (TFC)

A modified protocol of the Aluminium Chloride colorimetric method was followed with reference to Rejab and Ksibi,2019. To 100.0µl of each plant extract, 0.50ml of distilled water was poured and 150µl of 5% NaNO2 was added. The samples were incubated for 5 minutes and 250.0µl of 2% AlCl3 was added. After 6 minutes, 250.0µl of 1M NaOH was added, the solution was stirred vigorously and incubated for 10 minutes. The absorbance was measured in triplicates at 510nm and distilled water was used as the blank. TFC was calculated in mg Rutin equivalent/g (mgRE/g) with reference to the standard curve for Rutin.

Determination of the Total Antioxidant Capacity (TAC)

A modified protocol of the Phosphomolybdenum method was followed with reference to Biney et al., (2020). The reagent solution was prepared and 300.0µl of the reagent was mixed with 3.0ml of the reagent. The reaction solution was incubated at 95°C for 90 minutes in water-bath. Absorbance was measured in triplicates at 695nm and distilled water was used as the blank. TAC was calculated in mg Ascorbic acid equivalent/ g (mgAAE/g) with reference to the Ascorbic acid standard curve

Determination of the free radical scavenging activity

ABTS radical scavenging assay

ABTS assay was proceeded with reference to the methodology adopted from Shahinuzzaman et al., (2020) with some modifications. The ABTS reagent was prepared and incubated for 16 hours in the dark. To 100µl of the extract, 3.9ml of the reagent was mixed. The samples were incubated for 6 minutes at room temperature and the absorbance was measured in triplicates at 745nm using 100% ethanol as blank and 2.0ml of ABTS solution+1.0ml of methanol as the control. The absorbances were taken in triplicates and the scavenging activity was obtained by the following equation.

$$\text{ABTS scavenging activity} = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100\%$$

FRAP assay

The assay was adopted from Rajurkar and Hande, 2011 and few modifications were carried out. The FRAP reagent was prepared and 100.0µl of the plant extract was mixed with 1.90ml of FRAP. The samples were incubated for 30 minutes at room temperature. The absorbance was measured in triplicates at 620nm and FRAP reagent+ distilled water was used as the control. The scavenging activity was obtained by the following formula.

$$\text{FRAP} = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100\%$$

DPPH assay

The methodology of DPPH assay was adopted from Biney et al., 2020 and few modifications were carried out. A mass of 5.320mg of DPPH was mixed with 100.0ml methanol to prepare the reagent. To 1.0ml of the extract, 1.0ml of DPPH reagent was added. The mixture was inverted several times and was incubated in the dark for 30 minutes. The absorbance was measured in triplicates at 517nm and Ascorbic acid was used as the control. The values were measured in triplicates for each sample and the scavenging activity was obtained by the following equation.

$$\text{DPPH} = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100\%$$

RESULTS

Results of the Qualitative Analysis

The following table 4.0 summarizes the results obtained in the phytochemical screening tests of the plant extracts. The (+) sign depicts the positive results while (-) sign indicates the negative results

Table 4.0 Results of phytochemical tests

Phytochemical Test	Plant Extracts							
	Ethanol		Methanol		Chloroform		Distilled Water	
	Leaves	*Bark*	*Leaves*	*Bark*	*Leaves*	*Bark*	*Leaves*	*Bark*
Tannins	+	-	+	-	-	-	+	-
Saponins	-	-	+	-	+	+	+	-
Cardiac glycosides	-	+	-	+	-	-	-	+
Steroids	-	+	-	-	-	-	+	+
Terpenoids	+	-	+	+	+	+	-	-
Flavonoids	+	+	+	+	+	+	+	+
Phenols	+	-	-	-	-	-	-	-
Coumarins	+	+	+	+	+	-	-	+
Quinones	+	-	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+
Fats	-	-	-	-	-	-	+	-
Phytosterols	-	-	-	-	-	-	+	+
Phlobotannins	-	-	-	-	-	-	-	-

Results of the antioxidant capacity assays

The TPC values, expressed as µg GAE/g in Figure 3.0 were obtained from the Gallic Acid standard curve, (y = 0.0201x-0.3675 with a R2=0.974) where y represented the mean absorbance and x represented the concentration of gallic acid (µg/mL).

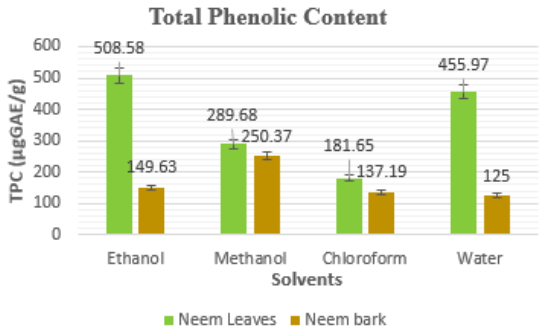


Figure 3.0 Total Phenolic Content of neem leaf and bark extracts with reference to Gallic Acid standard curve

The total flavonoid content of the samples expressed as mgRE/g in Figure 4.0 were obtained from the Rutin standard curve, (y = 0.1021x+0.0687 with a R2=0.9455) where y represented the mean absorbance while x showed the concentration of Rutin (mg/ml).

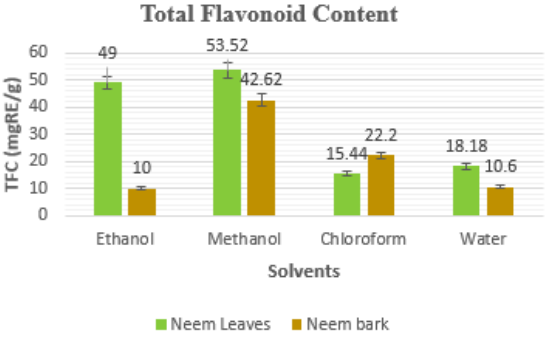
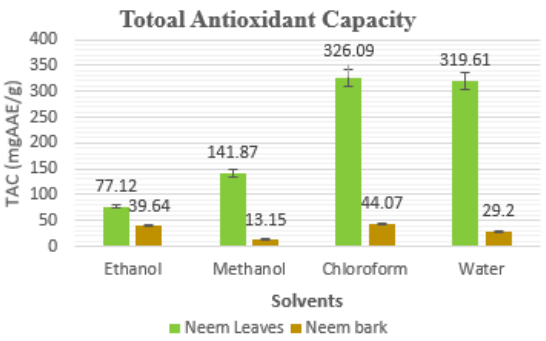


Figure 4.0 Total Flavonoid Content of neem leaves and bark extracts with reference to the Rutin standard curve.

The total antioxidant capacity values of the extracts, expressed as mgAAE/g in

Figure 5.0 below were derived with reference to the ascorbic acid standard curve (y = 0.0972x-0.0326 with a R2=0.9942), where y refers to the mean absorbance while x refers to the concentration of ascorbic acid (mg/ml).

Figure 5.0 Total Antioxidant Capacity of neem leaf and bark extracts with reference to Ascorbic acid standard curve.



Results of the free radical scavenging activity

The following figures 6.0, 7.0 and 8.0 illustrates the results of ABTS, FRAP and DPPH radical scavenging activities of the solvent extracts

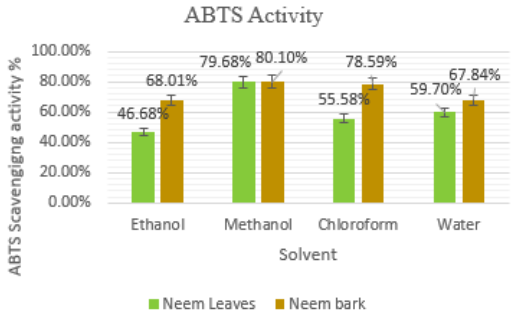


Figure 6.0 ABTS activity expressed by neem leaf and bark extracts

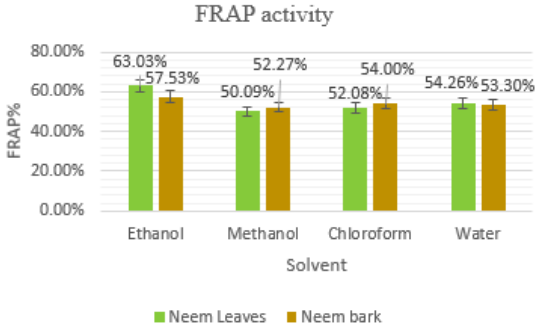


Figure 7.0 FRAP Activity expressed by neem leaf and bark extracts

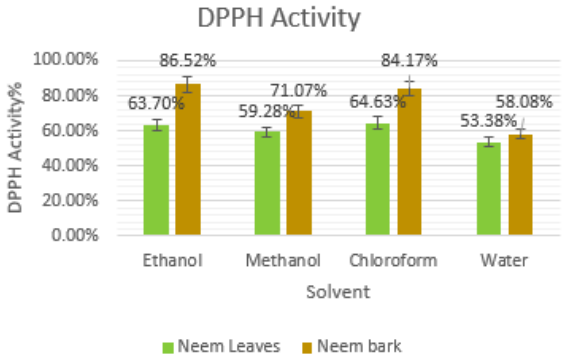


Figure 8.0 DPPH Activity expressed by neem leaf and bark extracts

DISCUSSION

Phytochemicals extracted from plant materials possesses antioxidants, which are effective in developing novel pharmaceutical strategies (Dhakal et al.,2016) Neem is one kind of a plant, considered as a rich source of a diverse variety of phytochemicals (Srivastava et al.,2020). This study was performed to examine the presence of phytochemicals and the antioxidant capacity of them with respect to four different solvent extractions of leaves and bark of Neem, underpinning various assays. But the

design of the study did not allow to recognize the fact that phytochemicals give rise to some of the tested properties such as free radical scavenging activity.

With reference to the results of the qualitative analysis in table 4.0 it is proven that most of Neem solvent extracts contained carbohydrates, flavonoids, coumarins and quinones which are supported by the study of Nelson et al., (2018). Same study reports flavonoids as one of the most popular secondary metabolites, which is proven by the results for flavonoids in this present study. Sharma and Patel (2018) states that methanolic extracts of both Neem leaf and stem-bark do not contain Phlobotannins which is proven by all the plant extracts of this study. Particularly Neem bark has revealed the absence of tannins which was consistent with the study of Sharma et al., (2014). According to Nelson et al., (2018) the presence of steroids in the stem bark and the absence of steroids in leaf extracts gives evidence as to why Neem bark is often used in pharmacotherapy in the production of reproductive hormones. Even though ethanolic and methanolic leaf extracts have detected carbohydrates in the present study, according to Shu'aibu et al., (2015) carbohydrates have not been detected in Neem leaves. The same study says such variations of phytochemicals could occur due to ecological and geographical factors in different study areas. According to the table 4.0 overall Neem leaves have reported in a higher number of phytochemicals than the bark. The positive results of neem leaves are supported by the studies of Ramadass and Subramanian, (2018) and Dash et al., (2017).

The selection of a solvent, substantially impacts on the yield of the extract as solubility levels of different compounds varies among different solvents. Therefore, selection of a solvent is a key parameter in both qualitative and quantitative analysis of different compounds (Biney et al., 2020).

According to the results of TPC on Figure 3.0 ethanol has proven a high yield of phenolic content in leaves than rest of the solvents and similar results were revealed in the studies of Do, et al., (2014) and Hismath et al., (2011). Ethanol is less polar in nature and it results in dissolution of low or non-polar constituents (Wangcharoen and Morasuk, 2007) which revels that the phenolic compounds that are responsible for the TPC of Neem leaves are less polar (Hismath et al.,2011) and a higher yield can be extracted from Ethanol. Highest TPC in Neem bark (Figure 3.0) was shown in methanol extracts which is similar with results of and Dhakal et al., (2016) and Sultana et al., (2009). It proves that phenolic compounds responsible for TPC in Neem bark possess moderately polar phenol groups than of leaves (Do et al., 2014) that is highly soluble in methanol. It is significantly shown that leaves have a higher TPC than in bark which is conflicting in, Abdulkadir et al., (2017) with reference to Neem but reveled in Abeysekera et al., (2013) for Cinnamon.

Many flavonoid compounds are strong antioxidants that effectively scavenge reactive oxygen species due to their phenolic hydroxyl groups (Anokwuru et al.,2011). As shown in Figure 4.0 in leaves, even though methanol has resulted a highest flavonoid content, its error bars overlap with ethanol leaf extracts resulting that both solvents have an equal capacity in extracting flavonoid compounds of Neem leaves. Methanol has reported the highest TFC values in Neem bark and it is approved in the results of Kiranmai et al., (2012). This can be attributable to flavonoids that gives rise to TFC in the Neem bark are highly soluble in methanol than in other three solvents. This indicates that Methanol is the best solvent for the extraction of flavonoids in Neem bark. According to the results significantly a

higher TFC is seen in Neem leaves than in Neem bark which is consistent with the results for TFC in the study of Abdulkadir et al., (2017).

When optimizing the values of TAC in Figure 5.0 it is clearly visible that chloroform and distilled water has shown an equal role in extracting antioxidant compounds in Neem leaves. Even though the quantity is shown in less, the highest amount of TAC values in Neem bark is shown by chloroform as the error bar is not overlapping with the rest. Similar results have been obtained in the study by Hossain et al., (2013). Compounds such as (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol, methyl 14-methylpentadecanoate, lineoleoyl chloride, phytol, methyl isoheptadecanoate and nonacosane are reveled to be responsible for high TAC in the chloroform extracts as mentioned in the study of Hossain et al., (2013). When comparing the results, leaves have proven to have a much higher antioxidant capacity than in bark extracts.

As shown in Figure 6.0 ABTS scavenging activity is significantly high in methanolic extracts of leaves than the rest of the leaf extracts. Both chloroform and distilled water leaf extracts has shown as an equal and intermediate scavenging activity as the error bars overlaps with one another Both methanol and chloroform has resulted in a high ABTS scavenging activity in bark and as the error bars are overlapping with each other, it is revealed that both has an equal impact towards the ABTS radical scavenging activity. But according to the results obtained by Kiranmai et al., (2012) chloroform has resulted in a very high ABTS scavenging activity while methanolic extracts have resulted in a much less scavenging activity. Moreover, ethanol and distilled water also has equal capabilities towards the ABTS scavenging activity in bark.

FRAP activity (Figure 7.0) was identified to be high in ethanol extracts of leaves while the other three solvent extracts were identified to have a similar contribution. These results are significantly different to the results of Kiranmai et al., (2012) as methanol has resulted in a higher FRAP scavenging activity compared to ethanol, chloroform, and ethyl acetate. In the same study chloroform has not shown FRAP activity which is conflicting from the results of the present study. All the four solvent extracts have an equal contribution towards FRAP scavenging activity of Neem bark as the error bars overlaps with one another which is again different from the results of Kiranmai et al., (2012)

In the present study both chloroform leaf and bark extracts has resulted in a similar DPPH scavenging activity along with the ethanol leaf and bark extracts as their error bars overlap with each other. According to Nahak and Sahu, et al., (2010) ethanol has the highest DPPH scavenging activity in different parts of Neem compared to water and methanol. The bark has resulted in a higher DPPH scavenging activity than the leaves in this study, proving the results of Abdulkadir et al., (2017).

CONCLUSION

Qualitative analysis in the present study confirms that Neem leaves and bark are rich with a variety of secondary metabolites out of which Neem leaves are confirmed to have a higher number of phytochemicals. Therefore, leaves have proven to have the highest antioxidant capacity. The study suggests that the antioxidant capacity and the antioxidant potential of a plant material can be measured with reference to its total phenolic content and total flavonoid content and by measuring the scavenging activity. The results, declare that the solvent of extraction depends on the compound that is to be extracted in determining the antioxidant capacities. Therefore, different solvents can be used

to extract different compounds from the plant extracts.

As future works, the impact of a more variety of solvents can be observed proceeding the same research to analyze the impact of different solvents towards the extraction of plant materials. Isolation, purification, characterization and structural elucidation of phytochemicals present in different parts of Neem using High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) and Nuclear Magnetic Resonance Spectroscopy (NMR) are recommended to be examine. Tests can be developed to detect antibacterial properties of Neem leaves and bark and statistical analysis can be performed to find the correlation between the antioxidant capacity and the antibacterial properties which can be aided in future drug discovery and pharmaceutical applications of the industry.

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REFERENCES

Abeysekera, W.P.K.M., Premakumara, G.A.S. and Ratnasooriya, W.D. (2013) "In Vitro Antioxidant Properties of Leaf and Bark Extracts of Ceylon Cinnamon (Cinnamomum zeylanicum Blume)", Tropical Agricultural Research, Vol.24, No.2, pp.128-138 [Online]. Available at: https://www.pgia.ac.lk/files/Annual_congress/journel/v24_2/Papers/2-_23_-W.P.K.M.pdf (Accessed: 1 September 2020).

Abdulkadir, A.R., Nashriyah, M. and Jahan, M.S. (2017) "In-vitro Antioxidant Potential in Leaf, Stem and Bark of Azadirachta indica" Tropical Agriculture, Vol. 40, No.4, pp.497-506, ResearchGate [Online]. Available at: https://www.researchgate.net/publication/322977309_In-vitro_Antioxidant_Potential_in_Leaf_Stem_and_Bark_of_Azadirachta_indica (Accessed: 2 September 2020).

Agrawal, D.P. (2002) "Medicinal properties of Neem: New Findings", Infinityfoundation. Available at: https://www.infinityfoundation.com/mandala/t_es/t_es_agraw_neem.htm (Accessed: 21 April 2020).

Ali, S., Khan, M.R., Irfanullah, Sajid, M. and Zahra, Z. (2018) "Phytochemical investigation and antimicrobial appraisal of Parrotiopsis jacquemontiana (Decne) Rehder", BMC Complementary and Alternative Medicine, Vol.18, No.43, January, BMC. [Online]. DOI: https://doi.org/10.1186/s12906-018-2114-z (Accessed 26 July 2020).

Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O. and Okebugwu, P. (2011) "Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant

activities of Three Nigerian Medicinal Plants", Nature and Science, Vol. 9, No. 7, ResearchGate [Online] Available at: https://www.researchgate.net/publication/267327206_Effect_of_Extraction_Solvents_on_Phenolic_Flavonoid_and_Antioxidant_activities_of_Three_Nigerian_Medicinal_Plants (Accessed 8 February 2021)

Bandiola, T.M.B. (2018) "Extraction and Qualitative Phytochemical Screening of Medicinal Plants: A Brief Summary", International Journal of Pharmacy, Vol. 8, No. 1, pp.137-143, ResearchGate. [Online] Available at: https://www.researchgate.net/publication/324674203_Extraction_and_Qualitative_Phytochemical_Screening_of_Medicinal_Plants_A_Brief_Summary (Accessed: 26 July 2020).

Biney, E.E., Nkoom, M., Darkwah, W.K. and Puplampu J.B. (2020) "High-performance liquid chromatography analysis and antioxidant activities of extract of Azadirachta indica (Neem) leaves" Pharmacognosy Research, Vol. 12, No. 1, pp.29-34. [Online] Available at: http://www.phcogres.com/article.asp?issn=0974-8490;year=2020;volume=12;issue=1;spage=29;epage=34;aulast=Biney (Accessed: 26 July 2020).

Braga, D.L., Mota, S.T.S., Zóia, M.A.P., Lima, P.M.A.P., Orsolin, P.C., Vecchi, L., Nepomuceno, J.C., Fürstenau, C.R., Maia, Y.C.P., Goulart, L.R. and Araújo, T.G. (2018) "Ethanolic extracts from Azadirachta indica leaves modulate transcriptional levels of hormone receptor variant in breast cancer cell lines, "International Journal of Molecular Sciences", Vol. 19, No. 7, 1897, PMC [Online] DOI: 10.3390/ijms19071879 (Accessed: 26 July 2020).

Chaudhary, S., Kanwar, R.K., Sehgal, A., Cahill, D.M., Barrow, C.J., Sehgal, R. and Kanwar, J.R. (2017) "Progress on Azadirachta indica Based Biopesticides in Replacing Synthetic Toxic Pesticides", Frontiers in Plant Science, Vol. 8, No. 610, May [Online]. Available at: https://www.frontiersin.org/articles/10.3389/fpls.2017.00610/full (Accessed: 10 April 2020).

Dash, S.P., Dixit, S and Sahoo, S. (2017) "Phytochemical and Biochemical Characterizations from Leaf Extracts from Azadirachta Indica: An Important Medicinal Plant", Biochemistry and Analytical Biochemical, Vol. 6, No. 2, [Online] DOI: 10.4172/2161-1009.1000323 (Accessed: 2 September 2020).

Dhakal,S., Aryal,P., Aryal S.and Bashyal, D. (2016) 'Phytochemical and antioxidant studies of methanol and chloroform extract from leaves of Azadirachta indica A. Juss. in Tropical region of Nepal', Journal of Pharmacognosy and Phytotherapy., Vol. 5, No. 2, pp.203-208, ResearchGate [Online] Available at: https://www.researchgate.net/publication/311984364_Phytochemical_and_antioxidant_studies_of_methanol_and_chloroform_extract_from_leaves_of_Azadirachta_indica_A_Juss_in_Tropical_region_of_Nepal (Accessed: 19 July 2020).

Dharajiya, D., Pagi, N., Jasani, H. and Patel P. (2017) "Antimicrobial Activity and Phytochemical Screening of Aloe vera (Aloe barbadensis Miller)", International Journal of Current Microbiology and Applied Sciences, Vol. 6, No. 3, pp.2152-2162, ResearchGate [Online] Available at: https://www.researchgate.net/publication/314646545_Antimicrobial_Activity_and_Phytochemical_Screening_of_Aloe_vera_Aloe_barbadensis_Miller (Accessed: 26 July 2020).

Dhawan, D. and Gupta, J. (2017) "Comparison of Different Solvents for Phytochemical Extraction Potential from Datura metel Plant Leaves", International Journal of Biological Chemistry, Vol. 11, No. 1, pp.17-22, Science Alert [Online]. Available at: https://scialert.net/abstract/?doi=ijb

c.2017.17.22 (Accessed: 20 April 2020).

Dhruti, M., Bhavika, P and Meonis, P. (2016) "Studies on phytochemical constituents and antioxidant activity of Alstonia scholaris", International Journal of Life Sciences, Vol. 4, No. 4, pp.529-538 [Online] Available at: http://oaji.net/articles/2017/736-1486399174.pdf (Accessed: 26 July 2020).

Do, Q.D., Angkawijaya, A.E., Nquyen, P.L.T., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.H. (2014) "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica", Journal of Food and Drug Analysis, Vol. 22, No. 3, pp.296-302, ScienceDirect [Online] Available at: https://www.sciencedirect.com/science/article/pii/S1021949813001348 (Accessed: 16 August 2020).

Engwa, G.A. (2018) "Free Radicals and the Role of Plant Phytochemicals as Antioxidants Against Oxidative Stress-Related Diseases", Intechopen, November, ResearchGate [Online]. DOI: 10.5772/intechopen.76719 (Accessed: 20 April 2020).

Fong, L.O., Berenguer, R.C., De La Vega, A.J., Wawoe, D.N. and Puente, Z.E. (2014) "Antioxidant potential of an aqueous leaf extract of Neem (Azadirachta Indica A. Juss)", Revista Cubana de Plantas Medicinales, Vol. 19, No. 2, pp.205-207, medigraphic.com [Online]. Availble at: https://www.medigraphic.com/cgi-bin/new/resumenI.cgi?IDARTICULO=53773 (Accessed: 20 April 2020).

Hismath, I., Wan Aida, W.M. and Ho, C.W. (2011) "Optimization of extraction conditions for phenolic compounds from neem (Azadirachta indica) leaves", International Food Research Journal, Vol. 18, No. 3, pp.931-939. [Online] Available at: http://www.ifrj.upm.edu.my/18%20(03)%202011/(11)IFRJ-2011-210.pdf (Accessed: 29 August 2020).

Hossain, M.A., Al-Raqui, K.A.S., Al-Mijizy, Z.H. and Weli, A.M. (2013) "Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris", Asian Pacific Journal of Tropical Biomedicine, Vol. 3, No. 9, pp.705-710. [Online] DOI: 10.1016/S2221-1691(13)60142-2 (Accessed: 29 August 2020).

Kasote, D.M., Katyare, S., Hegde, M.V. and Bae, H. (2015) "Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications", International Journal of Biological Sciences, Vol. 11, No. 8, pp.982-991, June, ResearchGate. [Online] DOI: 10.7150/ijbs.12096 (Accessed: 20 April 2020).

Kiranmai, M., Usha, S.B., Ibrahim, M. and Kumar, M.C.B. (2012) "Antioxidant activity andtotal flavonoids content of different parts of Azadirachta indica A. Juss", Journal of Medicinal Plants Research, Vol. 6, No. 46, pp.5737-5742. [Online] DOI: 10.5897/JMPR12.7 (Accessed: 29 August 2020).

Kumar, V.S. and Navaratnam, V. (2013) "Neem (Azadirachta indica): prehistory to contemporary medicinal uses to humankind", Asian Pacific Journal of Tropical Biomedicine, Vol. 3, No. 7, pp. 505-514, July, PubMed [Online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/23835719 (Accessed 2 April 2020).

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D. and Abete, P. (2018) "Oxidative stress, aging, and diseases", Clinical Interventions in Aging, Vol. 13, pp. 757-772, April, PMC [Online]. DOI: 10.2147/CIA.S158513 (Accessed: 2 April 2020).

Lobo, V. Patil, A. Phatak, A. and Chandra, N. (2010) "Free radicals, antioxidants and functional foods: Impact on human health", Pharmacognosy Reviews, Vol. 4, No. 8, pp.118-126,

PubMed. [Online] DOI: 10.4103/0973-7847.70902 (Accessed: 20 April 2020)

Maragathavalli, S., Brindha, S., Kaviyarasi, S. and Annadurai, B. (2012) "Antimicrobial Activity in leaf extract of Neem (Azadirachta Indica Linn.)", International Journal of Science and nature, Vol. 3, No. 1, pp. 110-113, ResearchGate [Online]. Available at: https://www.researchgate.net/publication/284377658_Antimicrobial_activity_in_leaf_extract_of_neem (Accessed: 2 April 2020).

Nahak, G. and Sahu, R.K. (2010) "Antioxidant activity in bark and roots of Neem (Azadirachta indica) and Mahaneem (Melia azedarach)", Continental Journal of Pharmaceutical Sciences 4, pp. 28 – 34 [Online] Available at: https://www.researchgate.net/publication/215613224_Antioxidant_activity_in_bark_and_roots_of_Neem_Azadirachta_indica_and_Mahaneem_Melia_azedarach (Accessed: 2 September 2020).

Nawaz, H., Shad, M.A., Rehman, N. and Andaleeb, H. (2020) "Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds", Brazilian Journal of Pharmaceutical Sciences, Vol. 56: e17129, ResearchGate [Online]. DOI: 10.1590/s2175-97902019000417129 (Accessed: 27 July 2020).

Nelson, N.O., Alexander, I., Okolie, J.E., Ernest, E., Ugwudike, P.O., Rita, O.N., Ezenwali, M.O., Odo, I.A., Ani, P.N. and Okolie, S.O. (2018), "Comparative analysis of the phytochemical compositions of leaf, stem-bark and root of Azadirachta Indica (Neem)", Universal Journal of Pharmaceutical Research, Vol. 3, No. 5, ResearchGate [Online]. DOI: 10.22270/ujpr.v3i5.201 (Accessed: 31 August 2020).

Pisoschi, A.M., Pop, A., Cimpeanu, C. and Predoi, G. (2016) "Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review", Oxidative Medicine and Cellular Longevity, Vol. 2016, Article ID 9130976, pp.1-36, ResearchGate [Online] DOI: 10.1155/2016/9130976 (Accessed: 2 April 2020).

Rajurkar, N.S. and Hande, S.M. (2011) "Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants", Indian Journal of Pharmaceutical Sciences, Vol. 73, No. 2, pp.146-151. [Online] DOI: 10.4103/0250-474x.91574 (Accessed: 27 July 2020).

Ramadass, N and Subramanian, N. (2018) "Study of phytochemical screening of neem (Azadirachta indica)", International journal of Zoology studies, Vol. 3, No. 1, pp.209-212 [Online]. Available at: http://www.zoologyjournals.com/archives/2018/vol3/issue1/3-1-49 (Accessed 28 August 2020).

Rejab, A. and Ksibi, H. (2019) "Phenolic and Flavonoid Contents of Some Plant Extracts from Tunisia Southern Landscape by Using Different Extraction Techniques: The Case of Retama reatam", Medicinal & Aromatic Plants, Vol. 8, No.5. [Online] Available at: https://www.longdom.org/open-access/phenolic-and-flavonoid-contents-of-some-plant-extracts-from-tunisia-southern-landscape-by-using-different-extraction-techniques-th-44673.html (Accessed: 27 July 2020).

Sahrawat, A., Sharma, J., Rahul, S. N., Tiwari, S., Joshi, D., Pundhir, A., Kumar, R., Akansha, Radhika, Bhavya, Shivani, Sharib, Nilesh, Govind and Akash (2018) "Phytochemical analysis and Antibacterial properties of Azadirachta indica (Neem) leaves extract against E.coli", Journal of Pharmacognosy and Phytochemistry, Vol. 7, No. 4, pp.1368-1371, ResearchGate [Online] Available at: https://www.researchgate.net/publication/337077726_Phytochemical_analysis_and_Antibacterial_properti

es_of_Azadirachta_indica_Neem_leaves_extract_against_Ecoli (Accessed 29 November 2020)

Sharma, A. and Patel, S. (2018) "Preliminary phytochemical screening and quantitative analysis of secondary metabolites of Mentha arvensis and Azadirachta indica", International Journal of Advanced Research and Development, Vol. 3, No. 1, pp. 114-118, ResearchGate [Online] Available at: https://www.researchgate.net/publication/333238353_Preliminary_phytochemical_screening_and_quantitative_analysis_of_secondary_metabolites_of_Mentha_arvensis_and_Azadirachta_indica (Accessed: 1 September 2020).

Sharma, Y., Dua, D. and Nupur, S.S. (2014) "Comparative study of different parts of Azadirachta Indica (Neem) plant on the basis of Antibacterial activity, Phytochemical screening and its effect on rat pc-12 (Pheochromocytoma) cell line", International journal of Biotechnology and Allied Fields, Vol. 2, No. 7, pp.144-154, ResearchGate [Online] Available at: https://www.researchgate.net/publication/289505237_COMPARATIVE_STUDY_OF_DIFFERENT_PARTS_OF_AZADIRACHTA_INDICA_NEEM_PLANT_ON_THE_BASIS_OF_ANTIBACTERIAL_ACTIVITY_PHYTOCHEMICAL_SCREENING_AND_ITS_EFFECT_ON_RAT_PC-12_PHEOCHROMOCYTOMA_CELL_LINE (Accessed: 1 September 2020).

Shahinuzzaman, M., Yaakob, Z., Anuar, F.H., Akhtar, P., Kadir, N.H.A., Hasan, A.K.M., Sobayel, K., Nour, M., Sindi, H., Amin, N., Sopian, K. and Akhtaruzzaman, M. (2020) "In vitro antioxidant activity of Ficus carica L. latex from 18 different cultivars", Scientific Reports, Vol. 10, 10852 [Online] Available at: https://www.nature.com/articles/s41598-020-67765-1#citeas (Accessed: 27 July 2020).

Shu'aibu, I., Hamman, J.B., Goje, L.J., Mu'inat, A.M., Jauro, H.A. and Kabiru, M.Y. (2015) "Phytochemical Analysis and Evaluation of bacteriostatic effect of Neem (Azadirachta Indica) leaves on some clinical bacterial isolates", Journal of Harmonized Research, Vol. 3, N0. 3, pp.152-157, ResearchGate [Online] Available at: https://www.researchgate.net/publication/318868153_Phytochemical_analysis_and_evaluation_of_bacteriostatic_effect_of_neem_azadirachta_indica_leaves_on_some_clinical_bacterial_isolates (Accessed: 7 February 2021).

Sonboli, A., Mojarrad, M., Ebrahimi, S.N. and Enayat, S. (2010) "Free Radical Scavenging Activity and Total Phenolic Content of Methanolic Extracts from Male Inflorescence of Salix aegyptiaca Grown in Iran", Iranian Journal of Pharmaceutical Research, Vol. 9, No. 3, pp. 293-296, PubMed [Online] Available at: https://www.researchgate.net/publication/259450389_Free_Radical_Scavenging_Activity_and_Total_Phenolic_Content_of_Methanolic_Extracts_from_Male_Inflorescence_of_Salix_aegyptiaca_Grown_in_Iran (Accessed: 27 July 2020).

Srivastava, S.K., Agrawal, B., Kumar, A. and Pandey, A. (2020) "Phytochemicals of Azadirachta Indica source of active medicinal constituent used for cure of various diseases: a review", Journal of Scientific Research, Vol. 64, No. 1, pp.365-390. [Online] Available at: https://www.bhu.ac.in/research_pub/jsr/Volumes/JSR_64_01_2020/53.pdf (Accessed: 31 August 2020).

Sultana, B., Anwar, F. and Ashraf, M. (2009) "Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts", Molecules, Vol. 14, No. 6, pp.2167-2180, PubMed [Online]. DOI: 10.3390/molecules14062167 (Accessed: 31 August 2020).

Sun, C., Wu, Z., Wang, Z. and Zhang, H. (2015) "Effect of Ethanol/Water Solvents on

Phenolic Profiles and Antioxidant Properties of Beijing Propolis Extracts", Evidence-Based Complementary and Alternative Medicine, Vol. 2015, Article ID 595393, Hindawi [Online]. Available at: https://www.hindawi.com/journals/ecam/2015/595393/ (Accessed: 30 March 2020).

Truong, D.H., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H. and Nguyen, H.C. (2019) "Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia", Journal of Food Quality, Vol. 2019, Article ID 8178294, Hindawi [Online] Available at: https://www.hindawi.com/journals/jfq/2019/8178294/ (Accessed: 20 April 2020).

Tütem, E., Apak, R. and Başkan, K.S. (2014) "Off-Line HPLC Integrated to Total Antioxidant Capacity Measurement of Beverages", Processing and Impact on Antioxidants in Beverages, pp. 265-276, [Online] Available at: https://doi.org/10.1016/B978-0-12-404738-9.00027-1 (Accessed: 20 April 2020).

Wilson, D.R. (2019) Medical News Today. Available at: https://www.medicalnewstoday.com/articles/325048 (Accessed: 30 March 2020).

Wangcharoen, W. and Morasuk, W. (2007) "Antioxidant capacity and phenolic content of holy basil" Songklanakarin Journal of. Science and Technology, Vol. 29, No. 5, pp.1407-1415. [Online] Available at: http://rdo.psu.ac.th/sjstweb/journal/29-5/0125-3395-29-5-1407-1415.pdf (Accessed: 26 July 2020).

Wangcharoen, W. and Morasuk, W. (2007) "Antioxidant capacity and phenolic content of some Thai culinary plants", Maejo International Journal of Science and Technology, Vol. 1, No. 2, pp.100-106, ResearchGate [Online]. Available at: https://www.researchgate.net/publication/284255562_Antioxidant_capacity_and_phenolic_content_of_some_Thai_culinary_plants (Accessed: 26 July 2020).

Yadav, D.K., Bharitkar, Y.P., Chatterjee, K., Ghosh, M., Mondal, N.B. and Swarnaka, S. (2016) "Importance of Neem Leaf: An insight into its role in combating diseases", Indian Journal of Experimental Biology, Vol. 54, No. 11, pp. 708-718, November, PubMed [Online]. Avaialble at: https://www.ncbi.nlm.nih.gov/pubmed/30179391 (Accessed: 18 April 2020).

Yadav, M., Chatterji, S., Gupta, S.K. and Watal, G. (2014) "Preliminary phytochemical screening of six medicinal plants used in traditional medicine", International Journal of Pharmacy and Pharmaceutical sciences, Vol. 6, No. 5, pp. 539-542, January, ResearchGate [Online]. Available at: https://www.researchgate.net/publication/286484827_Preliminary_phytochemical_screening_of_six_medicinal_plants_used_in_traditional_medicine (Accessed: 18 April 2020).

Yusuf, A.Z., Zakir, A., Shemau, Z, Abdullahi, M. and Halima, S.A. (2014) "Phytochemical analysis of the methanol leaves extract of Paullinia pinnata linn", Journal of Pharmacognosy and Phytotherapy, Vol. 6, No. 2, pp.10-16, February [Online]. DOI: 10.5897/JPP2013.0299 (Accessed: 18 April 2020).