

Comparative Assessment of Phytochemical Content and Antioxidant Potential of *Azadirachta indica* and *Parquetinanigrescens* Leaves

ABSTRACT

Aim: The aim of this study is to compare the phytochemical content and antioxidant potential of *Azadirachta indica* and *Parquetinanigrescens* leaves.

Study Design: This study was made to fit a one way Analysis of Variance.

Place and Duration of Study: This research was carried out in Premedical Department, Educational Advancement Centre, Ibadan and Pharmaceutical Laboratory of the University of Ibadan, Nigeria between January and June, 2018.

Methodology: Both plants were harvested from the botanical garden, University of Ibadan. The qualitative and quantitative analyses as well as antioxidant potential of both plants were investigated. The result of the qualitative analysis showed that both plants contained variety of the phytochemicals.

Results: The quantitative analyses showed that these phytochemicals are present in different concentrations. The concentration of phytate and total phenolics were significantly higher in *A. indica* when compared with those of *P. nigrescens* respectively at $p < 0.05$. It was also observed that *A. indica* had lower concentrations in alkaloids, saponin, flavonoids and tannin when compared with those of *P. nigrescens* respectively. Also tested for were antioxidants (ascorbic acid, DPPH and FRAP). The concentration of ascorbic acid was significantly higher in *A. indica* when compared with that of *P. nigrescens* at $p < 0.05$. α, α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging potential of *A. indica* and *P. nigrescens* was investigated respectively at different concentrations with *A. indica* having the higher radical scavenging potential. The scavenging potential of DPPH was found to increase with increasing concentration of the extracts.

Conclusion: Result of this shows that both plants are rich in phytochemicals and possess antioxidant potential. Hence, they might act as prophylactic and remedy for different diseases, such as cancer, atherosclerosis, obesity, etc. *Parquetinanigrescens* might be more potent than *Azadirachta indica* in acting as a remedy for different diseases.

Keywords: *Azadirachta indica*, *Parquetinanigrescens*, Phytochemical Content, Antioxidant Potential

1. INTRODUCTION

Azadirachta indica (neem) belongs to the family Meliaceae. It originated from South Asia, but grows widely in India, Pakistan and other tropical and sub-tropical parts of the world [1]. The tree was introduced into Nigeria from Ghana, and it was first grown from the seeds in Maiduguri, in the then Bornu Province (now Borno State),

Nigeria, in 1928. Neem plant was nicknamed 'DogonYaro' in Nigeria after a Neem tree nursery caretaker in Maiduguri who happened to be the first Neem tree caretaker in Maiduguri [2].

Neem is a fast-growing tree that can grow to a height of 35–40 m. It is evergreen, but in periods of drought it may shed most or almost all of its leaves. The Neem tree is noted for its drought

resistance. Neem seed pulp is useful for methane gas production. Its wood is used to make furniture. The neem tree has been used as a traditional remedy in ayurvedic medicine in India since antiquity, and medicinal properties have been especially ascribed to the leaves, fruit, and bark. The seed oil has been used for antimalarial, febrifuge, antihelminthic, vermifuge, and antiseptic and antimicrobial purposes, for bronchitis control, and as a healing agent against various skin disorders [3, 4]. Neem Oil is generally recommended for skin diseases while neem leaves are used for beauty purposes. The Neem leaf extracts have a powerful antiseptic, antifungal, antiviral and anti-bacterial effect unlike synthetic chemicals that often produce side effects such as allergic reactions, rashes etc. Neem is gentle and does not create any complications. Unlike Neem seed oil, Neem leaves have a pleasant odour. An extract from neem leaves can be prepared as an alcoholic tincture or as tea. The alcohol extract has a dark green colour and is effective for several weeks. It can be used in anti-ageing nourishing formulas, mouthwashes, facewashes, shower gels, soothing gels, face masks, skin toners etc. Another important pharmacological use of neem materials is as a dentifrice, reputedly producing remarkable healing of gum inflammations and paradontosia. Stomatitis is also known to be cured by an extract from bark of the neem tree [5]. One use of neem oil is in the manufacture of soap. The process was patented in India, and a hand soap containing neem fatty acids is now manufactured [6].

Parquetinanigrescens is a shrub found in equatorial West Africa has been in traditional medicine practice for centuries with its leaves, roots and latex all in use [7]. It is also known as bullock. It is perennial with twinning stem and woody base shortly tapering 10-15 cm long, 6-8 cm broad with a smooth long stem on the leaves. Bullock belongs to the family Asclepiadaceae. In Nigeria, the leaves have been reputed for treatment of helminthiasis (intestinal worm), while the roots are used for the management of rheumatism [8]. Over the years, *Parquetinanigrescens* has been used as an ingredient in the medications for insanity [9], as well as an aphrodisiac in East Africa. Other uses include the decoction of the stem bark been given as cardiac tonic while the leaf and root decoction have been used for the treatment of gonorrhoea and menstrual disorders [9]. *Parquetinanigrescens* is also a constituent of a commercial herbal preparation (Jubiformular) in Nigeria used in the treatment of anaemia in humans.

Previous studies by Mohammad, [10], Gayatri and Sahu, [11] and Emran *et al.* [12] reported the therapeutic role, antioxidant activity and analgesic, phytochemical and anti-inflammatory properties of *Azadirachta Indica* respectively. While Omoboyowa *et al.* [13] and Adu-Amoha *et al.* [14] carried out the phytochemical and hematological, toxicological studies of *Parquetinanigrescens*.

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [15]. They protect plants from disease and damage and contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals [16, 17]. Recently, it has been discovered that these compounds play important roles in human health when ingested into the body. Dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices [17]. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources [18]. Phytochemicals can be found in different parts of the plants, such as the leaves, flowers, roots, stems, seeds and fruits. Phytochemical concentration varies from plant to plant depending on the variety, growth conditions etc. These compounds are plants secondary metabolites. Plants produce these chemicals to protect themselves but it has been discovered that these compounds can protect humans against diseases. Depending on their role in plant metabolism, phytochemicals are classified as either primary or secondary constituents. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignins, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides [19]. Phenolics have been reported to be the most abundant and structurally diverse plant phytochemicals [20, 21].

An antioxidant can be defined as any substance that when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate [22]. The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving

free radicals. In recent years, a substantial body of evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases including atherosclerosis, chronic renal failure, and diabetes mellitus [23]. Since both plants have been reported to possess biological, pharmacological and radical-scavenging potentials, this study is aimed at comparing the potentials of both plants and identifies the most potent.

2. METHODOLOGY

2.1. Plant Preparation

Fresh matured leaves of both plants (*Azadirachta indica* and *Parquetina nigrescens*) were harvested from the Botanical garden of the University of Ibadan, Nigeria and were identified by a botanist, Mr. Abraham Adekale. The leaves were removed from the stem, washed and dried in the oven at a temperature of 37°C to remove moisture. The dried leaves were milled into powder by blending to increase the surface area for extraction.

2.2. Method of Extraction

The powdered leaves were extracted by soaking for 72 hours in enclosed glass jars (desiccators) using the cold method of extraction. Solvent used for both powdered leaves was ethanol. The solvent was evaporated using rotary evaporator at 37°C.

2.3. Qualitative Analyses of Phytochemicals

Qualitative determination of alkaloid, saponin, tannin, flavonoid, Phenol, steroids, anthraquinone glycosides, were carried by the methods described by Chandrashekaret al. [24], Carbohydrate was determined qualitatively by using Molisch's test [25], protein was carried out using Xanthoproteic test [25], anthocyanin, Coumarin, Emodins, phlobatannins were determined by the method described by Ashvinef al. [25], while terpenoid was determined qualitatively by using Salkowski's test [26].

2.4. Quantitative Analyses of Phytochemicals

Among the phytochemicals determined qualitatively, six present in both plants were analyzed quantitatively in triplicate. Saponin determination was carried out quantitatively by the

method of Obadoni and Ochuko [27], tannins were determined by Folin-Ciocalteu method [28], The concentration of total phenolics was determined spectrophotometrically, alkaloids and phytate were carried out by the method of Harborne [29], flavonoid was carried out by the method of Bohm and Kocipai-Abyazan [30].

2.5. Determination of Antioxidant

Ferric-Ion Reducing Antioxidant Power (FRAP) was determined in triplicate by assessing the ability of the extract to reduce FeCl₃ solution as described by Oyaizu [31], concentration of ascorbic acid was determined in triplicate by the iodimetry method [32], The antioxidant activity was measured in triplicate in terms of hydrogen donating or radical scavenging ability using the 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Method [33].

2.6. Statistical Analysis

Data were subjected to analysis using Graph Pad Prism, version 6.0. Results were presented as mean ± standard deviations. One way Analysis of Variance (ANOVA) was used for comparison of the mean. Differences between means were considered to be significant at p < 0.05 (95% confidence level).

3. RESULT

Table 1: Qualitative Analysis of Phytochemical content of *A. indica* and *P. nigrescens*

Phytochemicals	<i>A. indica</i>	<i>P. nigrescens</i>
Alkaloid (Hager's Test)	+	+
Saponin (Foam Test)	+	+
Anthraquinone (Borntrager's Test)	-	-
Tannin (Braymer's Test)	+	+
Phlobatannin (Precipitate test)	-	+
Anthocyanins	-	-
Terpenoid	-	-
Flavonoid	+	+
Phenols	+	+
Emodin	-	-
Coumarin	-	+
Glycosides (Liebermann's Test)	+	-
Steroid (Salkowaski Test)	-	+
Carbohydrate	-	-

(Molisch's Test)
 Protein - -
 (Xanthoproteic Test)

superscript along the same row are said to be significant at p<0.005.

Table 2: Concentrations of Phytochemicals in both Plants

Phytochemicals	<i>A. indica</i>	<i>P. nigrescens</i>
Saponin (%)	0.0050 ± 0.0002 ^a	0.0093 ± 0.006 ^b
Tannins (mg/g)	11.51 ± 0.385 ^a	16.5 ± 0.2 ^b
Total Phenolics (mg/g)	9.19 ± 0.1 ^a	7.71 ± 0.2 ^a
Alkaloids (%)	0.0123 ± 0.0006 ^a	0.0363 ± 0.0006 ^b
Flavonoid (%)	0.02 ± 0.005 ^a	0.03 ± 0.004 ^b
Phytic Acid (%)	1.1536 ± 0.019 ^a	0.972 ± 0.016 ^a

Results are presented as mean ± standard deviation where n=3. Values with different

Table 3: Concentrations of Ascorbic Acid and Ferric-ion Reducing Antioxidant Power

Antioxidant	<i>A. indica</i>	<i>P. nigrescens</i>
Ascorbic Acid (mg/g)	26.42 ± 2.14 ^a	17.61 ± 2.01 ^b
FRAP (mg/g)	315.25 ± 23.81 ^a	378.58 ± 31.15 ^b

Results are presented as mean ± standard deviation where n=3. Values with different superscript along the same row are said to be significant at p<0.005. FRAP = Ferric-ion Reducing Antioxidant Power

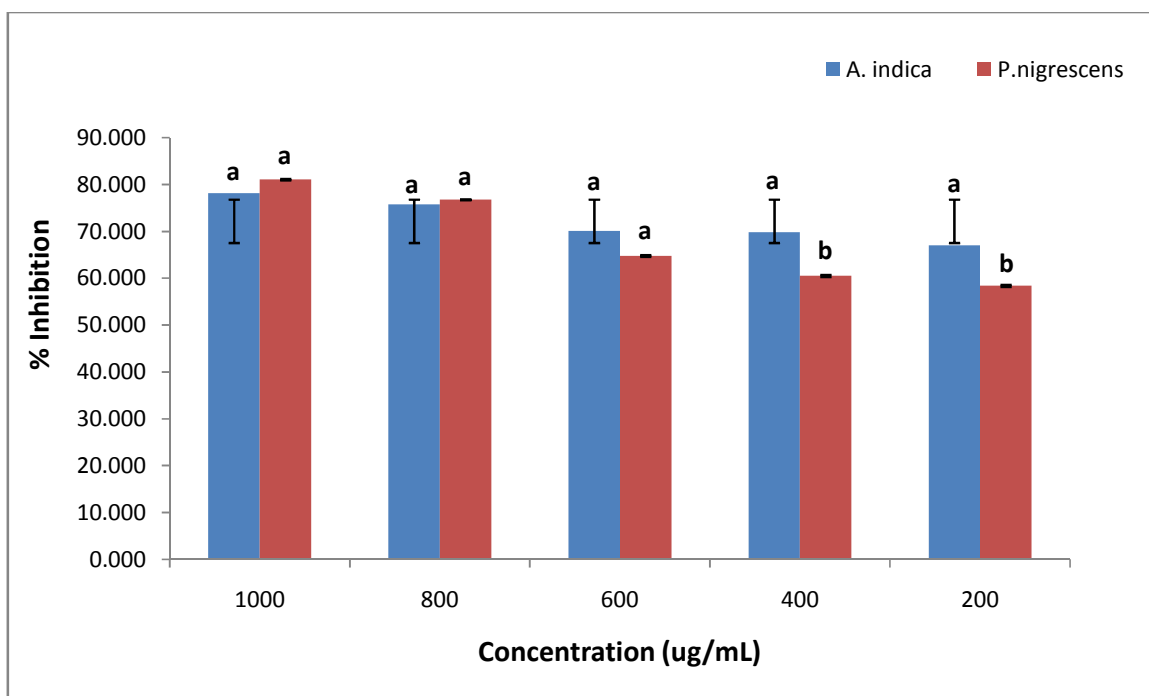


Figure 1: α, α-diphenyl-β-picrylhydrazyl (DPPH) Radical Scavenging potential of *A. indica* and *P. nigrescens* respectively at different concentrations. The result is presented as mean ± standard deviation with n = 3. Bars of the same concentration with different letters are significantly different at p<0.05.

4. DISCUSSION

Phytochemicals are chemicals produced by plants through secondary metabolism. They generally

have biological activities in the plant host and play a role in plant growth or defense against predators, pathogens or competitors [34]. They are commonly found in fruits, vegetables, nuts, legumes, and grains. Phytochemicals include all plant compounds both plant chemicals that are beneficial and those that are toxic. Some phytochemicals possess incredible health benefits while others are toxic to health [35].

In this study, it was observed that the concentration of phytic acid of *A. indicaw* was significantly higher when compared with that of *P. nigrescens* at $p < 0.05$. Research carried out on populations consuming vegetable and plant diet rich in phytates has shown lower incidence of cancer, which suggests that phytate, has an anticarcinogen effect [36, 37]. The metal binding characteristics of phytate endowed it an antioxidant function, inhibiting the production of hydroxyl radicals that normalize cell homeostasis [38] and it also serves as a natural food antioxidant [39]. Therefore both plants might have anticarcinogenic properties but *Azadirachtaindica* might be more potent when compared with *Parquetinanigrescens*.

Thompson [40] also suggested that dietary phytate may also be beneficial for diabetic patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying. Phytate has also been shown to regulate insulin secretion [41]. It is believed that phytate decreases blood clots, cholesterol and triglycerides and thus prevents heart diseases [42]. Both plants might have the propensity of being natural remedies for the treatment of diabetes mellitus but *Azadirachtaindica* might be more potent when compared with *Parquetinanigrescens*.

It has also been reported that Phytic acid prevents renal stone development [43, 44]. Wise [45] through research discovered that it has the ability as a complexing agent to remove traces of heavy metal ions from the kidney. It prevents calcium oxalate precipitation in the kidney and reduces oxalate excretion in renal stone patients. Calcium oxalate crystal deposition *in vitro* on urothelium is prevented by phytic acid by protecting the membrane from free radical-mediated damage [45]. This might makes *Azadirachtaindica* potentially better in preventing renal stone and removing traces of metal ions than *Parquetinanigrescens*.

It was observed in this study that the concentration of alkaloids of *Azadirachtaindica* was significantly lower when compared with that of *Parquetinanigrescens* at $p < 0.05$. Alkaloids are

natural products that contain heterocyclic nitrogen atoms. They are basic in character [46]. Alkaloids are known for different biological activities and each activity has its own specific mechanism of action. D-tubocurarine is one such example of alkaloids that possesses the antiparalytic activity due to its ability to obstruct the acetylcholine receptor spots which enable the muscles to unwind at neuromuscular intersections [47]. Both plants might have antiparalytic activity but *Perquetinanigrescens* might be more active than *Azadirachtaindica*. Alkaloids also possess antioxidant property and anticancer activity due to their ability to act as scavenger of free radicals, metal chelating activity or electron or hydrogen donation ability. These alkaloids have also been reported to exert chemopreventive effect against tumour cells by terminating or causing depolymerisation of protein microtubules that forms the mitotic spindle in cell division. This results in hindrance in the process of division and separation of tumour cells and reduces the incidences of cancer. This is in support of the research carried out by Moura *et al.* [48] who reported the ROS scavenging ability, antimutagenic and antigenotoxic activities of betacarboline alkaloids, found in medicinal plant and variety of foods. *Parquetinanigrescens* might therefore have higher potential of having chemopreventive effect than *Azadirachtaindica*.

In this study, it was observed that the concentration of Saponins of *Azadirachtaindica* was significantly lower when compared with that of *Parquetinanigrescens* at $p < 0.05$. Saponins are naturally occurring surface-active glycosides with a distinctive foaming characteristic. They are mainly produced by plants. Saponin has been reported by Surana *et al.* [49] to have effect in hemolysis. The hemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for the phospholipids present in the cell membrane with which they form insoluble complexes. Saponins have a lytic action on erythrocyte membranes. This can either be beneficial or of negative effect. Prior to hemolysis, erythrocytes may enter suicidal cell death (apoptosis), thus leading to clearance of defective erythrocytes prior to release of hemoglobin [50]. According to Bissinger *et al.* [51] exposure of human erythrocytes to saponin stimulates Ca^{2+} entry with subsequent triggering of cell membrane scrambling and thus suicidal death of human erythrocytes. The effect is paralleled by hemolysis. This in turn leads to anemia and thrombosis. The presence of significant saponin in both plants might make them to have the propensity to make more erythrocytes available but *A. indicam* might be better in this than *P.*

nigrescens. Saponin has also been reported to have effect in cholesterol metabolism as it lowers serum cholesterol levels. Large mixed micelles formed by the interaction of saponins with bile acids account for their increased excretion. The resulting accelerated metabolism of cholesterol in the liver causes its serum levels to go down [52]. This might make *P. nigrescens* a better natural remedy for disease conditions such as obesity, cardiovascular diseases and other cholesterol related diseases than *A. indica*.

Saponin has also been reported to possess hypolipidaemic activity. The mechanism involved in the hypolipidemic activity is that saponin has high fiber content. The fiber significantly binds to cholesterol hence aiding its excretion [53]. It has anti-inflammatory properties. The significant ameliorative activity of the saponins may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin along with its antioxidant property which inhibits the formation of ROS which also plays a major role in inflammation (Sayyahet *et al.*, 2004). *P. nigrescens* might have a higher potential in hypolipidaemic and anti-inflammatory activities when compared to *A. indica*. The negative effect of saponins on animal reproduction has long been reported and has been ascribed to their abortifacient, antizygotic and anti-implantation properties. Saponins are found to be extremely strong stimulators of luteinising hormone release from cultured hypophysial cells [55]. The saponins show antimicrobial activity by inhibiting the growth of Gram positive and Gram negative microorganisms. Some saponins are not effective against Gram negative microorganisms because they are unable to penetrate into the cell membranes of the microorganisms [56, 57]. This might make *A. indica* have higher propensity of antimicrobial activity when compared to *P. nigrescens*.

In this study, it was also observed that the concentration of flavonoids of *A. indica* was significantly lower when compared with that of *P. nigrescens* at $p < 0.05$. As natural antioxidants, flavonoids play an important role in scavenging free radicals and preventing degenerative diseases such as cardiovascular diseases [58, 59, 60]. However, they are also involved in the antiproliferation of carcinogenic cells, in cell cycle regulation and in the induction of apoptosis [61, 62, 63]. They can act to inhibit free-radical mediated cytotoxicity and lipid peroxidation, as anti-proliferative agents to inhibit tumor growth or as weak estrogen agonists or antagonists to modulate endogenous hormone activity [57]. In these ways, they may confer protection against

chronic diseases such as atherosclerosis and cancer and assist in the management of menopausal symptoms. They contain conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants *invitro* or cell free systems by scavenging superoxide anion, singlet oxygen, lipid peroxyradicals, and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species [60]. This is in agreement with the research carried out by [64], who studied 83 prostate cancer patients and 107 age-matched controls. They reported that after adjusting total calories, high increased consumption of most phytoestrogens, including isoflavones and other flavonoids, had to a small degree a protective effect on prostate cancer risk. Also, coumestrol (a phytoestrogen, mimicking the physiological actions of estrogens and estradiol), daidzein, and genistein showed the strongest protective associations. Several studies have reported the potential of some plants extracts to prevent peptic ulcer due to the presence of flavonoid [65, 66, 67]. *P. nigrescens* might therefore be a better natural remedy for treatment of diseases such as cardiovascular diseases, cancer and atherosclerosis as well as prevention of peptic ulcer when compared to *A. indica*.

It was observed that the concentration of tannin of *A. indica* was significantly lower when compared with that of *P. nigrescens* at $p < 0.05$. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers [26, 68]. Tannins possess wound healing activity by its ability to increase the collagen content, which is one of the factors for promotion of wound healing [69]. This might make *P. nigrescens* better in wound healing process than *A. indica*. Tannin is a non-toxic compound and they can generate physiological responses in animals that consume them [70]. Tannin can be toxic to filamentous fungi, yeast and bacterial. The presence of tannin in both plants under study might suggest the ability of these plants to play key roles as antifungal, antibacterial, antidiarrheal, antioxidant and antihemorrhoidal agent [71].

In this study, it was observed that the concentration of total phenolics of *A. indica* was higher when compared with that of *P. nigrescens* at $p < 0.05$. Plant phenolics are one of the secondary metabolites from plants with a variety of pharmacological and functional properties. These phenolic compounds neutralize reactive oxygen species or free radicals by donating a hydrogen atom or an electron chelating metal ion in aqueous solutions [72]. The phenolic compounds extracted from plants possess

multiple biological properties such as antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, and anti-mutagenic properties, related to functional groups present on each phenolic compound [73]. It could be that phenolic compounds (where flavonoids is one of the main class), are known to be hydrophilic antioxidants and are the most abundant secondary metabolite in plants [74].

Another essential part of this study is antioxidant studies. An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate [75]. One important function of antioxidants toward free radicals is to suppress free radical-mediated oxidation by inhibiting the formation of free radicals and/or by scavenging radicals. The formation of free radicals may be inhibited by reducing hydroperoxides and hydrogen peroxide and by sequestering metal ions [76] through complexation/chelation reactions. Radical scavenging action is dependent on both reactivity and concentration of the antioxidant.

α , α -diphenyl- β -picrylhydrazyl (DPPH) test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test [77]. The percentage inhibition of the two plants, *A. indica* and *P. nigrescens* was investigated with *A. indica* having higher radical scavenging potential. Scavenging of DPPH radical was found to increase with increasing concentration of the extracts (Figure 1). Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [78]. Oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders such as inflammation, viral infections, autoimmune pathologies, and digestive system disorders including gastrointestinal inflammation and ulcer. For instance in diabetes, increased oxidative stress which co-exist with reduction in the antioxidant status has been postulated: Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein,

inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long term complication of diabetes [77]. Similarly, in carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma. Hence, therapy using free-radical scavenging antioxidants has potential to prevent, delay or ameliorate many of these disorders [78]. *A. indica* might have a higher propensity of DPPH radical scavenging potential when compared with *P. nigrescens*.

It was observed in this study that the concentration of ascorbic acid of *A. indica* was significantly higher when compared with that of *P. nigrescens* at $p < 0.05$. Ascorbic acid is involved in many physiological functions in living organisms. Notably, low plasma levels of vitamin C were associated with death from cardiovascular disease (CVD) [79] and it has been speculated in literature that vitamin C may protect against CVD through several mechanisms. Vitamin C enhances endothelium-dependent vasodilatation, thereby preventing endothelial dysfunction associated with atherosclerosis, hypercholesterolemia, hypertension, diabetes and smoking. This process seems to involve the ability of vitamin C to increase the atheroprotective nitric oxide (NO) [80]. Thus vitamin C was shown to enhance the activity of endothelial NO synthase by keeping its cofactor, tetrahydrobiopterin, in a reduced state and thereby increasing its intracellular availability [77, 78]. *A. indica* might therefore serve as a natural remedy for the prevention of CVD compared to *P. nigrescens*.

Its role in the synthesis of collagen in connective tissues is well known [81]. The absence of wound healing and the failure of fractures to repair are classically recognized features of scurvy. These features are attributable to impaired collagen formation due to lack of vitamin C. This might make *A. indica* a better natural remedy for the treatment of wound than *P. nigrescens*.

The possible use of vitamin C in cancer therapy and prevention has been an area of great interest. Thus it is tempting to speculate that vitamin C supplements, if able to prevent the formation and/or promote the repair of pre-mutagenic oxidative DNA lesions, could be of use in cancer prevention. In addition, an early report showed that daily supplementation with vitamin C at high doses (grams) increased the survival time of terminal cancer patients [82] and it was suggested

that vitamin C could have important anticancer properties [82]. Indeed, vitamin C kills or inhibits the growth of many tumour cell lines and potentiates the cytotoxicity of radiosensitising drugs [82]. There are also several reports showing that cancer cell lines are more sensitive to vitamin C than their non-malignant counterparts. Regarding cancer prevention, several epidemiological studies have linked the consumption of a diet rich in fruit and vegetables (and therefore in antioxidants) with lower incidence of many types of cancer [77]. *A. indica* might therefore have a higher potential of preventing cancer than *P. nigrescens*.

In this study, it was observed that the concentration of FRAP of *A. indica* was significantly lower when compared with that of *P. nigrescens* at $p < 0.05$. FRAP assay has many advantages over radical scavenging assays such as excellent reproducibility, linearity over a wide range and high sensitivity. In contrast, the FRAP assay measures the reducing capability by increased sample absorbance and the assay may not complete even several hours after the reaction starts, such that a single end point of the reaction cannot be determined [83]. FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom [84]. FRAP assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction. This might make *P. nigrescens* have better ferric-ion reducing potential antioxidant than *A. indica*.

5. CONCLUSION

In this study, it was observed that both plants are rich in phytochemicals and possess antioxidant potential. Hence, they might act as prophylactics and remedy to different diseases such as cardiovascular diseases, peptic ulcer, diabetes mellitus, etc. *P. nigrescens* will be more potent than *A. indica* as a prophylactic and remedy against diseases. Further studies at molecular level is recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- [1]. Bokhari, M. H. and Aslam K. M. Neem (*MeliaAzadirachta A. Juss*). A useful tree in Northern Nigeria. *Annals of Borno*. 1985;3: 83 – 86.
- [2]. National Research Council, *Neem: a tree for solving global problems*. National Academy Press, Washington, DC. 1992; 115pp.
- [3]. Chopra, I.C., Gupta, K. C., and Nazir, B. N. Preliminary study of antibacterial substances from *Meliasadirachta*. *Indian J. Med. Res.* 1952; 40: 511-515.
- [4]. Sinniah, D., and Baskaran, G. Margosa oil poisoning as a cause of Reye's syndrome. *Lancet*. 1981; 1:487-489.
- [5]. Lorenz, H. K. P. Neem tree bark extract in the treatment of inflammatory stomatitis. *Zahnaerztl. Praxis*. 1976;8: 1-4.
- [6]. Godrej, A. B. The fatty acid industry and minor oils. In *Proceedings of a workshop on minor oil seed collection, processing and end uses*. Calcutta, India, February 23, 1975. East India Oil Millers Association. 1975; pg. 101-102.
- [7]. Gill, L.S. Ethnomedical uses of plants in Nigeria. *University of Benin Press Publisher.*, 1992; pg 80-181.
- [8]. Adeyemi, S.O. Ethnobotanical study of the antirheumatic plants in parts of Oyo, Ogun and Lagos States. Project Report in the Department of Microbiology and Botany, University of Ibadan. 1994Pg 46.
- [9]. Iwu, M.M. Handbook of African Medicinal plants. *CRC Press, Boca Raton . FL*. 1993; 351
- [10]. Mohammad A. A. Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Hindawi Publishing Corporation*. 2016; pp 1-11.
- [11]. Rengasamy, S., Kaushik, N., Kumar, J., Koul, O. & Parmar, B.S. In: Singh RP (ed) *World Neem Conf.* Oxford and IBHCO, New Delhi., 1993; p 207
- [12]. Emran, T.B., Nasir, U.M.M., Rahman, A., Uddin, Z. and Islam, M. Phytochemical, Antimicrobial, Cytotoxic, Analgesic and Anti-Inflammatory Properties of

- AzadirachtaIndica: A Therapeutic Study. *J Bioanal Biomed*.2015 S12: 007.
- [13]. Omoboyowa D. A., Ogunneye A. L., Igara C. E. and Otuchristian G. Phytochemical screening and haematological studies of Parquetinagrescens ethanol and chloroform leaves extracts in normal albino rats. *African Journal of Pharmacy and Pharmacology*.2015; 10(10):164-169.
- [14]. Adu-Amoaha, L., Agyare, Christian.,Kisseih, E., Patrick, G. A. and Kwesi, B. M. Toxicity assessment of *Erythrophleumivorense* and *Parquetinagrescens*.*Toxicology Reports*. 2014;1 :411–420.
- [15]. Hasler, C.M. and Blumberg JB.. Symposium on Phytochemicals: Biochemistry and Physiology. *Journal of Nutrition*. 1999; 129: 756S-757S.
- [16]. Gibson, E.L., Wardel, J. and Watts, C.J. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. *Appetite*.1998; 31: 205-228.
- [17]. Mathai, K. Nutrition in the Adult Years In Krause's Food, Nutrition, and Diet Therapy. 10th ed., ed. L.K. Mahan and S. Escott-Stump.2000; 271: 274-275.
- [18]. Moorachian, M.E. Phytochemicals: Why and How? *Tastings*2000; pg 4-5.
- [19]. Hahn, N.I. Is Phytoestrogens Nature's Cure for What Ails Us? A Look at the Research.*Journal of the American Dietetic Association*.1998; 98: 974- 976.
- [20]. Yoshie, Y., Wang, W., Hsieh, Y. and Suzuki, T. Compositional difference of phenolic compounds between two seaweeds. *Halimeda spp. J. Tokyo Univ. Fish*.2001; 88: 21-24.
- [21]. Zakaria, N.A., Ibrahim, D., Sulaiman, S.F. and Supardy, A. Assessment of antioxidant activity, total phenolic content and in-vitro toxicity of Malaysian red seaweed, *Acanthoporphoraspcfifera*. *J. Chem. Pharm. Res*. 2011; 3: 182-191.
- [22]. Halliwell, B. and Gutteridge, J.C. The definition and measurement of antioxidants in biological systems.*Free RadicBiol Med*. 1995; 18:125–6.
- [23]. Young, I. S. and Woodside, J V. Antioxidants in health and disease. *J ClinPathol*.2001; 54:176–186.
- [24]. Chandrashekar, K., Santanu, S. and Prasanna K. Phytochemical studies of aerial parts of the plant *Leucaslavandulaefolia*. *Der PharmaChemica*.2010; 2 (5):434-437.
- [25]. Ashvin, G., Rajaram, S., & Ashok, S. Phytochemical analysis of ethanolic extract of roots of *carrisacaranduslinn*. *Rasayan j. Chem*. 2012; 5 (4): 456-459.
- [26]. Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C. and Atangbayila, T.O. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*.2008; 7 (3):1019–1024.
- [27]. Obadoni, B.O. and Ochuko, P.O. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci*.2001; 8 b:203-208.
- [28]. Rajeev, S., Pawan, K. V. and Gagandeep, S. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. *J IntercultEthnopharmacol*. 2012; 1(2):101-104.
- [29]. Harborne, J. Phytochemical methods. Chapman and Hall, Ltd London.1973; p.49-88.
- [30]. Bohm, B.A. and Kocipai- Abyazan, R. Flavonoid and condensed tannins from the leaves of *Vaccinumraticulation* and *Vaccinumcalcyimium*. *Pacific Sci*.1994; 48: 458-463.
- [31]. Oyaizu, M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Japan J. Nutr*. 1986; 44: 307-315.
- [32]. U.S.P. United States Pharmacopoeia (Twentieth Revision)..Mack Co., Easton PA, 1980; p.55.

- [33]. Charalampos, P., Konstantina, L., Olga K. M., Panagiotis, Z. and Vassileia, J. S. Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. *Antioxidants*.2013; 2: 11-22.
- [34]. Molyneux, R.J., Lee, S.T., Gardner, D.R., Panter, K.E. and James, L.F. Phytochemicals: the good, the bad and the ugly. *Phytochemistry*. 2007; 68(22-24): 2973- 85.
- [35]. Hennemen, K. Nutritional and health info-sheet: some facts about phytochemicals. UC Davis: Dept of nutrition, the regents of the University of California, Davis campus. 2016
- [36]. Shamsuddin, A. M. Anti-cancer function of phytic acid. *International Journal of Food Science and Technology*.2002; 37(7): 769–782.
- [37]. Vucenik, I., and Shamsuddin, A. M. Cancer inhibition by inositol hexaphosphate (IP6) and inositol: From laboratory to clinic. *Journal of Nutrition*. 2003; 133: 3778S–3784S.
- [3]. Minihane, A. M., and Rimbach, G. Iron absorption and the iron binding and antioxidant properties of phytic acid. *International Journal of Food Science and Technology*. 2002; 37(7): 741–748.
- [39]. Raboy, V. Seeds for a better future: Low phytate grains help to overcome malnutrition and reduce pollution. *Trends in Plant Science*.2001; 6:458–462.
- [40]. Thompson, L. U. Potential health benefits and problems associated with antinutrients in foods. *Food Research International*.1993; 26: 131–149.
- [41]. Barker, C. J., and Berggren, P. Inositol hexakisphosphate and beta-cell stimulus secretion coupling. *Anticancer Research*.1999; 19: 3737–3742.
- [42]. Onomi, S., Okazaki, Y., and Katayama, T. Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high-sucrose diet. *Bioscience Biotechnology and Biochemistry*.2004; 68: 1379–1381.
- [43]. Grases, F., Prieto, R. M., Simonet, B. M., and March, J. G. Phytate prevents tissue calcifications in female rats. *BioFactors*, 2000a; 11: 171–177.
- [44]. Grases, F., March, J. G., Prieto, R. M., Simonet, B. M., Costa-Bauza, A., Garcia-Raja, A. Urinary phytate in calcium oxalate stone formers and healthy people – Dietary effects on phytate excretion. *Scandinavian Journal of Urology and Nephrology*. 2000b; 34: 162–164.
- [45]. Wise, A. Blood lead levels after chronic feeding to mice of lead acetate with calcium phytate in the diet. *Bulletin of Environmental Contamination and Toxicology*.1982; 29: 550–553.
- [46]. Mueller-Harvey, I. and McAllan, A.B. Tannins. Their biochemistry and nutritional properties. In: *Advances in plant cell biochemistry and biotechnology. Morrison IM, ed. JAI Press Ltd, London (UK)*.1992; 1:151-217.
- [47]. Gustafson, T. Pharmacological control of muscular activity in the sea urchin larva. I. Effects of nicotinic and muscarinic agents. *CompBiochemPhysiol C: Comp Pharmacology*.1989; 94:1-14.
- [48]. Moura, D.J., Richter, M.F., Boeira, J.M., PegasHenriques, J.A. and Saffi, J. Antioxidant properties of beta-carboline alkaloids are related to their antimutagenic and antigenotoxicactivities. *Mutagenesis*.2007; 22:293-302.
- [49]. Surana, S.J., Tatiya, A.U., Jain, A.S., Desai, D.G., Shastri, K.V. and Katariya, M.V. Pharmacognostical and physiochemical standardization of root of

- Eranthemumroseum*(Vahl) R. Br. *Phcognosy Magazine*.2008; 4: 75-79.
- [50]. Waheed, A., Barker, J., Barton, S.J., Owen, C.P., Ahmed, S, Carwema. A novel steroidal saponinglycoside from *Fagoniaindica* induces cell- selective apoptosis or necrosis in cancer cells. *Eur J Pharm Sci*.2012; 47: 464-73.
- [51]. Bissinger, R., Paola, M., Kousi, A., Sabina, H., Caterina, F., Majed, A. and Florian L. Effect of Saponin On Erythrocytes. *Int J Hematol*. 2014; 100: 51-59.
- [52]. Oakenfull, D.G. Aggregation of bile acids and saponins in aqueous solution. *Australian Journal of Chemistry*.1986; 39: 1671-1683.
- [53]. Raju, J., Gupta, D., Rao, A., Yadava, P. and Baquer, N. *Trigonellafoenumgraecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. *Molecular and Cellular Biochemistry*.2001; 224: 45-51.
- [54]. Sayyah, M., Hadidi, N. and Kamalinejad, M. Analgesic and anti-inflammatory activity of *Lactuca sativa* seed extract in rats. *Journal of Ethnopharmacology*., 2004; 92: 325-329
- [55]. Benie, T., El-Izzi, A., Tahiri, C., Duval, J and Thieulant M.L.T. *Combretodendronafricanum* bark extract as an antifertility agent : Estrogenic effects in vivo and LH release by cultured gonadotrope cells. *Journal of Ethnopharmacology*.1990; 29: 13-23.
- [56]. Soetan, K.O., Oyekunle, M.A., Aiyelaagbe, O.O. and Fafunso, M.A. Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor* L. Moench. *African Journal of Biotechnology*.2006; 5(23): 2405-2407.
- [57]. Jain, A.S., Surana S.J., Gokhale S.B., Tatiya A.U and Bothara R.C. Antimicrobial properties of *Eranthemumroseum* (Vahl) R. Br. *IranianJournal of Pharmaceutical Research*. 2007; 6(2): 131-133.
- [58]. Cragg, G.M. and Newman, D.J. Natural products: A continuing source of novel drug leads. *Biochim.Biophys.Acta* . 2013; 1830: 3670–3695.
- [59]. Milella, L., Milazzo, S., De Leo, M., Vera Saltos, M.B., Faraone, I., Tuccinardi, T., Lapillo, M., De Tommasi, N. and Braca, A. α -Glucosidase and α -Amylase Inhibitors from *Arcytophyllumthymifolium*. *J. Nat. Prod*.2016; 79: 2104–2112.
- [60]. Bisio, A., De Mieri, M., Milella, L., Schito, A.M., Parricchi, A., Russo, D., Alfei, S., Lapillo, M., Tuccinardi, T. and Hamburger, M. Antibacterial and Hypoglycemic Diterpenoids from *Salvia chamaedryoides*. *J. Nat. Prod*. 2017; 80: 503–514.
- [61]. Ugartondo, V., Mitjans, M., Touriño, S., Torres, J.L. and Vinardell, M.P. Comparative antioxidant and cytotoxic effect of procyanidin fractions from grape and pine. *Chem. Res. Toxicology*.2007; 20: 1543–1548.
- [62]. Russo, D., Valentão, P., Andrade, P.B., Fernandez, E.C. and Milella, L. (2015). Evaluation of antioxidant, antidiabetic and anticholinesterase activities of *Smallanthussonchifolius* landraces and correlation with their phytochemical profiles.*Int. J. Mol. Sci*. 2007; 16: 17696–17718.
- [63]. Rue, E.A., Rush, M.D. and Van Breemen, R.B. Procyanidins: A comprehensive review encompassing structure elucidation via mass spectrometry. *Phytochem. Rev*. 2017; 1–16.
- [64]. Siess, M.H., Le Bon, A.M., Canivenec-Lavier, M.C., Amoit, M.J., Sabatier, S., Aubert, S.Y. and Suschetet, M. Flavonoid of honey and propolis: Characterisation and effects on hepatic drug-metabolizing enzymes and benzo[a]pyrene-DNA binding in rats. *J Agric Food Chem*. 1996; 40: 2297–2301.
- [65]. Airaodion, A. I., Obajimi, O.O, Ezebuoro, C.N., Ogbuagu, U., Agunbiade, A. P., Oloruntoba, A.P., Akinmolayan, J.D., Adeniji, A.R and Airaodion, E.O. Prophylactic Efficacy of Aqueous Extract of *Curcuma longa* Leaf Against Indomethacin-Induced Ulcer. *International Journal of Research*.2019; 6(1):87-91.
- [66]. Airaodion, A. I., Ogbuagu, U., Ogbuagu, E. O., Airaodion, E. O., Agunbiade, A. P.,

- Oloruntoba, A. P., Mokelu, I. P. & Ekeh, S. C. Investigation of Aqueous Extract of *Zingiber officinale* Root Potential in the Prevention of Peptic Ulcer in Albino Rats. *International Journal of Research and Innovation in Applied Science*. 2019;4(2):64-67.
- [67]. Airaodion, A. I., Olayeri, I. M., Ewa, A. O., Ogbuagu, E. O., Ogbuagu, U., Akinmolayan, J. D., Agunbiade, A. P., Oloruntoba, A. P., Airaodion, E. O., Adeniji, A. R., Obajimi, O. O. & Awosanya, O. O. Evaluation of *Moringa oleifera* Leaf Potential in the Prevention of Peptic Ulcer in Wistar Rats. *International Journal of Research*. 2019;6(2):579-584.
- [68]. Varahalarao, V. and Kaladhar, D.S.V.G.K. Antimicrobial study of plant extracts of *Daturametel* L. against some important disease causing pathogens. *Asian Pacific Journal of Tropical Disease*. 2012; S94-S97.
- [69]. Pandit, R., Phadke, A. and Jagtap, A. Antidiabetic effect of *Ficus religiosa* extract in streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*. 2010; 128: 462-466.
- [70]. McDevitt, J. T., Schneider, D. M., Katiyar S. K. and Edlind, F. S. Berberina: a candidate for the treatment of diarrhea in AIDS patients abstract. In program and Abstracts of the 36th Interscience conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D. C. 1996;
- [71]. Asquith T. N. and Butter L. G. Interaction of condensed tannins with selected proteins. *Phytochemistry journal*. 1986; 25:1591-1593.
- [72]. Nile, S.H and Park, S.W. Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds. *Ind Crop Prod*. 2015; 70:238-244.
- [73]. Shui, G. and Leong, L.P. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by highperformance liquid chromatography. *J Chromatogr A*. 2002; 977:89-96.
- [74]. Gil, M.I., Thomas, B. F.A., Hess-Pierce, B., Hplcroft, D.M. and Kader, A.A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agri Food Chem*. 2000; 48:4581-4589.
- [75]. Gutteridge, J.M.C Free Radicals and Aging. *Rev. Clin. Gerontol.*, 1994; 4, 279-288.
- [76]. Niki, E. Antioxidant Activity: Are We Measuring It Correctly? *Nutrition*. 2002; 18: 524-525.
- [77]. Duarte, T.L. and Lunec, J. Review: When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. *Free Rad. Res.* 2005; 39(7): 671-686.
- [78]. Huang, A., Vita, J.A., Venema, R.C. and Keaney, J.F. Ascorbic acid enhances endothelial nitric oxide synthase activity by increasing intracellular tetrahydrobiopterin. *J. Biol. Chem.* 2000; 275: 17399-17406.
- [79]. Khaw, K.T., Bingham, S., Welch, A., Luben, R., Wareham, N., Oakes, S. and Day, N. Relation between plasma ascorbic acid and mortality in men and women in Epic-Norfolk prospective study: A prospective population study. *Lancet*. 2001; 357: 657-663.
- [80]. May, J.M. How does ascorbic acid prevent endothelial dysfunction? *Free Rad. Biol. Med.* 2000; 28:1421-1429.
- [81]. Aguirre, R. and May, J.M. Inflammation in the vascular bed: Importance of vitamin C. *Pharmacol. Ther.* 2008; 119: 96-103.
- [82]. Cameron, E. and Pauling, L. Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. *Proc. Natl. Acad. Sci.* 1976; 73: 3685-3689.
- [83]. Shahat, A.A., Ibrahim, A.Y. and Alsaid, M.S. Antioxidant capacity and polyphenolic content of seven Saudi Arabian medicinal herbs traditionally used in Saudi Arabia. *Indian J Tradit Knowle.* 2015; 14 (1): 28-35.
- [84]. Duh, P., Du, P. and Yen, G. Action of methanolic extract of mung bean hull as inhibitors of lipid peroxidation and non-lipid

oxidative damage. *Food and Chem Toxicol.* 1999; 37:1055-1061.