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# Comparative Assessment of Phytochemical Content and Antioxidant Potential of *Azadirachta indica* and *Parquetina nigrescens* Leaves

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author AIA conceptualized and designed the study and also and wrote the draft of the manuscript. Authors APA, EOA and APO wrote the protocol. Authors POO, JDA and OOA performed the statistical analysis. Authors EOO and UO managed the literature searches. Authors ARA, OOO and OAA managed the analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** The aim of this study is to compare the phytochemical content and antioxidant potential of *Azadirachta indica* and *Parquetina nigrescens* 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 Science 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.
**Results:** The result of the qualitative analysis showed that both plants contained variety of phytochemicals. The quantitative analyses showed that these phytochemicals were 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 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 study showed 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. *Parquetina nigrescens* might be more potent than *Azadirachta indica* in acting as a remedy for different diseases.


*Keywords: Azadirachta indica; Parquetina nigrescens; 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 'Dogon Yaro' 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, face washes, 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].

*Parquetina nigrescens* 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 Ascelpiadaceae. 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, *Parquetina nigrescens* 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]. *Parquetina nigrescens* is also a constituent of a commercial herbal preparation (Jubi formular) 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-Amoaha et al. [14] carried out the phytochemical and hematological, and toxicological studies of *Parquetina nigrescens* respectively.

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 compound 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 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. Olukunle A. Adekale. The leaves were removed from the stem, washed and dried in the oven at a temperature of 37ºC 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 37ºC.

## 2.3 Qualitative Analyses of Phyto-chemicals

Qualitative determination of alkaloid, saponin, tannin, flavonoid, Phenol, steroids, anthraquinone glycosides, were carried by the methods described by Chandrashekar *et 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 Ashvin et al. [25], while terpenoid was determined qualitatively by using Salkowski's test [26].

## 2.4 Quantitative Analyses of Phyto-chemicals

Among the phytochemicals determined qualitatively, six present in both plants were analyzed quantitatively in triplicate.

### 2.4.1 Determination of saponin concentration

Saponin determination was carried out in triplicate by the method of Obadoni and Ochuko [27]. 20 g of each sample were put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml 20% aqueous ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of di-ethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded, the purification process was repeated. 60 ml of n-butanol was added, the combined n–butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath, after evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as a percentage.

Percentage (%) Saponin = $((W_2-W_1)$ / Weight of the Sample) × (100/1)

Where,

$W_1$ = Weight of evaporating dish
$W_2$ = Weight of evaporating dish + sample

### 2.4.2 Determination of tannin concentration

The tannins were determined in triplicate by Folin - Ciocalteu method. 0.1 ml of the sample solution was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% $Na_2CO_3$ solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an Spectrum lab 752s UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract [28].

**Calculation**

Tannic acid $(\frac{\text{mg}}{100\text{g}})$

Tannin as tannic acid= (C × Extract volume ×100/ Aliquot volume ×weight of sample)

Where, C is concentration of tannic acid.

### 2.4.3 Determination of total phenolic concentration

The concentration of phenolics in plant extracts was determined in triplicate using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7% Sodium carbonate ($Na_2CO_3$) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm

of extract [28]. The total phenolic contents in all samples were calculated using the formula:

$$C = \frac{cv}{M}$$

Where,

C = total phenolic content mg GAE/g dry extract,
C = concentration of sample obtained from calibration curve in mg/Ml,
V = volume of extract in ml,
M = mass of extract in gram.

#### 2.4.4 Determination of total alkaloids concentration

This was carried out in triplicate by the method of Harborne [29]. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [29]. The percentage of flavonoid was calculated as:

% Alkaloid = (Weight of Alkaloid/ Weight of Sample) ×100

#### 2.4.5 Determination of flavonoid concentration

This was carried out in triplicate by the method of Bohm and Kocipai Abyazan [30]. 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

The percentage of Flavonoid was calculated as

% Flavonoid = (Weight of Flavonoid/ Weight of Sample) ×100

#### 2.4.6 Determination of phytic acid concentration

Phytates were determined through phytic acid determination using the procedure described by Harborne [29]. 2.0 g of each sample were weighed into a 250 ml conical flask. Samples were soaked in 100 ml of 2% concentrated HCl for 3 hours and then filtered through a double layer Whatman No 1 filter paper. 50 ml of each of the sample filtrate were placed in a 250 ml beaker and 100 ml of distilled water was added. To each sample, 10 ml of 0.3% Ammonium thiocyanate indicator solution was added. Titrated then followed with standard iron chloride solution which contained 0.00195 g iron/ml. The end point was signified by the appearance of a brownish-yellow coloration that persisted for 5 min. The percentage phytic acid was calculated as follows:

% Phytic acid = y × 1.19 × 100

Where, y = titre value × 0.00195 g

### 2.5 Determination of Antioxidant

Ferric-Ion Reducing Antioxidant Power (FRAP) was determined in triplicate by assessing the ability of the extract to reduce $FeCl_3$ 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 $\pm$ 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. RESULTS

Both plants contained diverse phytochemicals as shown in Table 1.

**Table 1. Qualitative analysis of phytochemical content of *A. indica* and *P. nigrescens***

| Phytochemicals | *A. indica* | *P. nigrescens* |
|---|---|---|
| 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) | - | - |

**Table 2. Concentrations of phytochemicals in both plants**

| Phytochemicals | *A. indica* | *P. nigrescens* |
|---|---|---|
| Saponin (%) | $0.0050 \pm 0.0002^{a}$ | $0.0093 \pm 0.006^{b}$ |
| Tannins (mg/g) | $11.51 \pm 0.385^{a}$ | $16.5 \pm 0.2^{b}$ |
| Total Phenolics (mg/g) | $9.19 \pm 0.1^{a}$ | $7.71 \pm 0.2^{a}$ |
| Alkaloids (%) | $0.0123 \pm 0.0006^{a}$ | $0.0363 \pm 0.0006^{b}$ |
| Flavonoid (%) | $0.02 \pm 0.005^{a}$ | $0.03 \pm 0.004^{b}$ |
| Phytic Acid (%) | $1.1536 \pm 0.019^{a}$ | $0.972 \pm 0.016^{a}$ |

*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*

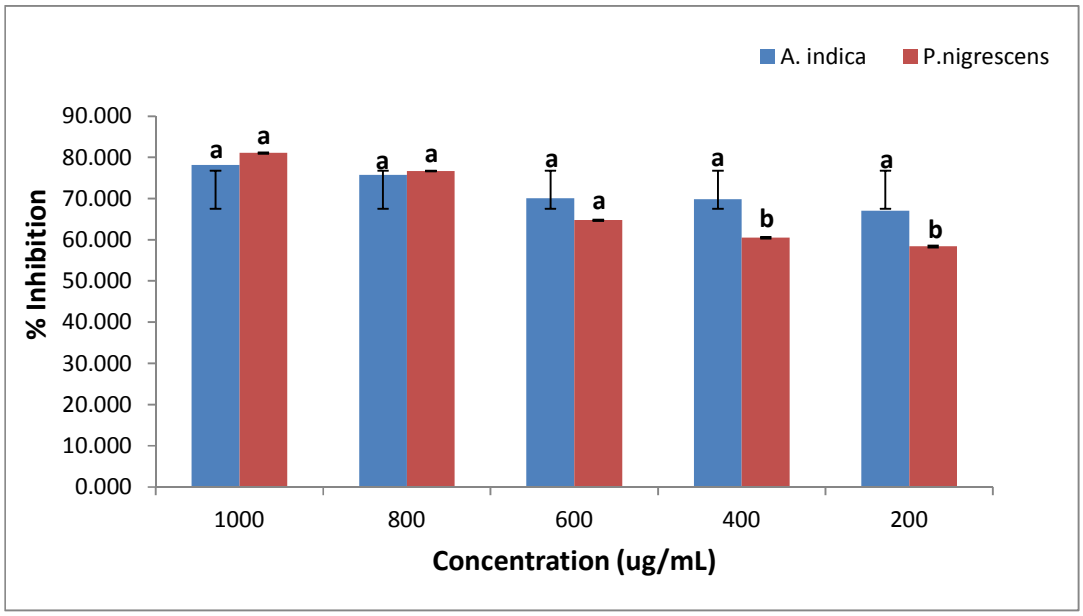


**Fig. 1. α, α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging potential of *A. indica* and *P. nigrescens* respectively at different concentrations. The result is presented as mean ± standard deviation with n = 3. Bars of the same concentration with different letters are significantly different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

**Table 3. Concentrations of ascorbic acid and ferric-ion reducing antioxidant power**

| Antioxidant | *A. indica* | *P. nigrescens* |
|---|---|---|
| Ascorbic Acid (mg/g) | $26.42 \pm 2.14^{a}$ | $17.61 \pm 2.01^{b}$ |
| FRAP (mg/g) | $315.25 \pm 23.81^{a}$ | $378.58 \pm 31.15^{b}$ |

*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*

## 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. indica* 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 *Azadirachta indica* might be more potent when compared with *Parquetina nigrescens.*

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 *Azadirachta indica* might be more potent when compared with *Parquetina nigrescens*.

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* urothelium is prevented by phytic acid by protecting the membrane from free radical-mediated damage [45]. This might makes *Azadirachta indica* potentially better in preventing renal stone and removing traces of metal ions than *Parquetina nigrescens.*

It was observed in this study that the concentration of alkaloids of *Azadirachta indica* was significantly lower when compared with that of *Parquetina nigrescens* 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 acetycholine receptor spots which enable the muscles to unwind at neuromuscular intersections [47]. Both plants might have antiparalytic activity but *Perquetina nigrescens* might be more active than *Azadirachta indica.* 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. *Parquetina nigrescens* might therefore have higher potential of having chemopreventive effect than *Azadirachta indica*.

In this study, it was observed that the concentration of Saponins of *Azadirachta indica*

was significantly lower when compared with that of *Parquetina nigrescens* 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. indica* 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 [54]. *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 micro-organisms. 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 *in vitro* 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 decolourize 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 (Fig. 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 radio sensitising 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 possesses 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. This pharmacological study is a useful tool for further drug development from the natural plant products. Further study at molecular level is recommended.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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