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THE USE OF FLAVONOIDS AS A MARKER FOR STANDARDIZATION OF *LONCHOCARPUS SERICEUS*

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ABSTRACT

The first part of this project presents the antioxidant and antimicrobial activity of the flavonoids from the leaves, root and bark of Lonchocarpus sericeus as a means of verifying folkloric use. The extraction of flavonoids from the various organs of the plant was done and then phytochemical screening tests to determine the presence of flavonoids, alkaloids, saponins, cardiac glycosides, terpenoids, anthraquinones and tannins performed. The synthesis of the pure flavonoid to serve as a marker compound and its functional group determination were as well carried out. The identification of the flavonoids in the plant was achieved and the antimicrobial and antioxidant activities of the various organs as well as the synthesized flavonoid established. It was established that a particular kind of flavonoid, chalcone was present in the stems, leaves and roots of Lonchocarpus. The chalcone was found to have both antimicrobial and antioxidant activities. The chalcone present in the plant samples was found to be of higher antioxidant activity than ascorbic acid, a standard antioxidant. This presents useful information towards the standardization of the various organs obtained from the plant.

KEYWORDS: Lonchocarpus sericeus Antioxidant Antimicrobial Chalcone Flavonoids Ascorbic acid.

1. NTRODUCTION

Plant secondary metabolism produces products that aid in the growth and development of plants but are not required for the plant to survive. Secondary metabolism facilitates the

primary metabolism in plants. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. [Gottfried F. S. 1959]. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, and recreational drugs. [Evans W.C., 2002].

There is no fixed, commonly agreed upon system for classifying secondary metabolites. Based on their biosynthetic origins, plant secondary metabolites can be divided into three major groups:

1. Flavonoids and allied phenolic and polyphenolic compounds,
2. Terpenoids and
3. Nitrogen-containing alkaloids and sulphur-containing compounds.

Other researchers have classified secondary metabolites into the following, more specific types;

Alkaloids, Non-protein amino acids (NPAAs), Amines, Cyanogenic glycosides, Glucosinolates, Alkamides, Lectins, peptides and polypeptides, Terpenes, Steroids and saponins, Flavonoids and Tannins, Phenylpropanoids, lignins, coumarins and lignans, Polyacetylenes, fatty acids and waxes, Polyketides, Carbohydrates and organic acids. [Bidlack W. R. 2000].

Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P or citrin. [Benthsath A. et al., 1937]. These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in coloring the plants. In addition, flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities. Flavonoids are also found to be powerful anti-oxidants and researchers are looking into their ability to prevent cancer and cardiovascular diseases. [Cazarolli L. H. et al., 2008]. Flavonoids help prevent cancer by inducing certain mechanisms that may help to kill cancer cells, and researches believe that when the body processes extra flavonoid compounds, it triggers specific enzymes that fight carcinogens. Good dietary sources of Flavonoids are all citrus fruits, which contain the

specific flavonoids; hesperidin, quercitrin and rutin, berries, tea, dark chocolate and red wine and many of the health benefits attributed to these foods come from the Flavonoids they contain. Flavonoids are synthesized by the phenylpropanoid metabolic pathway where the amino acid phenylalanine is used to produce 4-coumaryol-CoA, and this is then combined with malonyl-CoA to produce chalcones which are backbones of Flavonoids. [Ververidis F. F. et al., 2007]. Chalcones are aromatic ketones with two phenyl rings that are important in many biological compounds. The closure of chalcones causes the formation of the flavonoid structure. Flavonoids are also closely related to flavones which are actually a sub class of flavonoids, and are the yellow pigments in plants. In addition to flavones, 11 other subclasses of Flavonoids including, isoflavones, flavans, flavanones, flavanols, flavanolols, anthocyanidins, catechins (including proanthocyanidins), leukoanthocyanidins, dihydrochalcones, and aurones have been identified.

Lonchocarpus sericeus (Fabaceae), currently observed in savannahs throughout the tropical areas in the world, is a tree whose parts are used in association with other herbs or alone for medicinal purposes. It is usually a shrub or small tree but occasionally up to 16 m tall and 40 cm in diameter. It is mostly found in wet places in coastal forest but also in the dry forest types, gallery forests and savannas. It possesses a smooth bark; slash many layered with gritty streaks, yellow to brown, with red to black spots or lines of exudate, spongy. Its leaves are imparipinnate, 3 - 4 jugate; young buds covered with a yellow pubescence; reddish pubescent rachis; stipples absent; venation ± scalariform. Flowers are silvery yellow. Fruit has a flat pod of about 12 cm long, slightly contracted between the seeds, with 2 - 5 seeds.

A decoction or infusion of the leaves of the plant is used for gastrointestinal and hepatic disorders and for alleviation of malaria. It is also used in folk medicine for its antitumoral, antimicrobial, antiviral, antimycotic, choleretic-cholagog, hepatoprotector properties. The plant is also consumed as a diuretic and a tonic to maintain wellness. The root, the bark and the leaves of this plant is being used as an anti-inflammatory, antimicrobial and anticancer plant in some rural communities in Ghana.

Many diseases are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance [Fresquet, F. *et al*., 2006]. The antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radical [Vilioglu, Y.S., et al. 1998]. However, there have been concerns about synthetic antioxidants such as

butylated hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) because of their possible activity as promoters of carcinogenesis. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity [Barlow S.M., 1990]. Therefore, search for natural antioxidant has greatly been increased in the recent scenario.

Many studies have shown that natural antioxidants from plant sources can effectively inhibit oxidation of food and reduce the risk of age-dependent diseases (Burda S. & Oleszek W, 2001; Zou Y. *et al*. 2004). Flavonoids, abundant in fruits, vegetables, medicinal plants, have attracted the greatest attention and have been studied extensively, because they are a kind of highly effective antioxidants with a lower toxicity than synthetic antioxidants such as BHA and BHT (Pekkarinen S. S. *et al*. 1999).

In order to standardize plant samples there is the need to check the presence of the possible secondary metabolites in the plant samples. The standardization method used may be qualitative, quantitative or both. Standardization guarantees the presence and or content of one or more active constituents and marker compounds. Some standardization methods used include phytochemical screening tests, Thin Layer Chromatography, UV assay methods, and titrations.

This work is thus geared towards determining the presence of flavonoids in the root, bark and leaves of *Lonchocarpus sericeus*, to identify the specific flavonoid present in the plant samples and finally to establish the antimicrobial and antioxidant activity of the plant samples.

1.1 Aim

To verify the use of the root, bark and leaves of *Lonchocarpus sericeus* for antioxidant and antimicrobial activities and to investigate the possible use of chalcone as a means to quantify the flavonoids in all three parts of L. sericeus.

1.2 Objectives

1. To identify as far as possible the compound responsible for the antioxidant and antimicrobial activities in *Lonchocarpus sericeus*.

2. To conduct phytochemical screening test to compare the phytochemical profile of the various parts of *L. sericeus.*
3. To determine the antioxidant and antimicrobial activities of the roots, bark and leaves of *L. sericeus.*
4. To synthesize a flavonoid and use it as a tool for standardization.

1.3 Justification

Many studies have shown that natural antioxidants from plant sources can effectively inhibit oxidation and reduce the risk of age-dependent diseases.

Most rural folks in Ghana especially those in Ada and Kpong use the bark, root and leaves of *Lonchocarpus sericeus* for treating several diseases that are due to oxidative stress, hence the quest to investigate if all three parts of this plant possess antioxidant and antimicrobial properties.

The anti-oxidant properties are usually due to flavonoids and hence at attempt at standardization of plant material using flavonoids from the plant is possible.

2. METHODOLOGY

2.1 Plant Material

The leaves, bark and root of *L. sericeus* were collected in November 2014 from *Pankrono* at Kumasi, Ghana and identified by Mr. Asare, a herbalist at the Pharmacognosy department, Faculty of Pharmacy, KNUST, Kumasi.

The *roots and bark* were cut into small pieces and air-dried for 10 days. After drying, the fragments were ground into powder using Thomas Wiley Machine. The powdered samples were then packed in appropriate containers ready for flavonoid extraction.

The leaves were air-dried for 14 days. The dry samples were milled and ground into powder using Thomas Wiley Machine. Fatty constituents and chlorophyll from the powdered leaves were extracted with petroleum ether by soxhlet extraction. About two hours was taken to extract all fatty constituents and solvent became colorless when left for further extraction. The defatted sample was air dried for 12 hours then flavonoids extraction from defatted plant material was performed.

2.2 Extraction of Flavonoids

5.00g of defatted leaves were taken and shifted into filter paper thimble. 500mL of aqueous ethanol (70%) were poured into round bottom flask followed by fitting it on soxhlet assembly in a heating mantle. Extraction time was 5 hours, until the clear solution was observed in Soxhlet, where thimble was placed. Solvent was then evaporated under vacuum and the extract was heated in an oven (60°C) to dryness.

5.00g each of powdered bark and roots were taken and shifted into separate filter paper thimbles. 500mL of aqueous ethanol (70%) were poured into round bottom flask followed by fitting it on soxhlet assembly in a heating mantle. Extraction time was 3 hours, until the clear solution was observed in Soxhlet, where thimble was placed.

The ethanol extract was concentrated using a rotary evaporator at room temperature and heated in an oven (60°C) to dryness to obtain a dark green pigment.

2.3 Phytochemical screening

Phytochemical screening was performed using standard procedures [Sofowora A. 1993], [Evans W.C 2002].

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigourously and observed for a stable persistent froth.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for detection of flavonoids

Lead acetate test

Small quantity of extract + lead acetate solution =Yellow colour ppt.

Sodium hydroxide test

Plant extract + NaOH =yellow colour which decolorize after addition of glacial acetic acid.

Ammonia vapour test

A strip of filter paper is dipped in a flavonoid solution.

The filter paper is dried and exposed to ammonia vapour and the appearance of an intense yellow colour observed.

The yellow colour disappears when the strip of filter paper is exposed to fumes of HCl or dilute HCl.

Flavonoids contain the benzopyran nucleus and occur either as glycosides or as aglycones. The aglycones are pale yellow and their glycosides are colourless. Both forms when made alkaline give an intense yellow colour, which disappears in the presence of a mild acid or fumes of a strong acid.

2.4 The Antioxidant Assay

Scavenging of 2, 2 diphenyl-picrylhydrazyl (DPPH) radical assay

The free radical scavenging activity was determined as described by Govindarajan et al., (2003). 1 ml each of the different concentrations of the extracts and the synthesized chalcone (500 – 62.5 µg/ml) were added to 3ml methanolic solution of DPPH (20 mg/l) in a test tube. The reaction mixture was kept at $25^{O}C$ for 30 minutes. Process was repeated for concentrations of ascorbic acid with concentrations (50- 6.25 µg/ml). The absorbance of the residual DPPH was determined at 517 nm in UV-visible spectrophotometer. The DPPH radical scavenging activity was calculated according to the following equation:

% DPPH radical scavenging activity = $1 - [A_{sample}/A_{control}] \times 100$

Where A_{sample} and $A_{control}$ are absorbance of sample and control respectively. The concentration of sample required to scavenge 50% of DPPH is expressed as EC50 (Vani et al., 1997).

2.5 Preparation of reagents and extracts

2.5.1 Preparation of Ethanolic extracts of *Lonchocarpus sericeus*

0.01 g of the extract was weighed and dissolved in 20 ml of methanol to obtain solution strength of 500 µg/ml concentration. The other concentrations 250, 125 and 62.5µg/ml were prepared by serial dilution.

2.5.2 Preparation of DPPH solution

The 20mg/L DPPH solution was prepared as follows;

20 mg = 1000 ml

X mg = 100 ml

100ml x 20mg
1000ml
2 mg = 0.002 g

It is very inaccurate to weigh 0.002g hence 0.02g of DPPH was weighed and dissolved in 100ml of methanol to produce a concentration of 200mg/L. Serial dilution was then performed to make a concentration of 20mg/L.

C1V1=C2V2
200mg/L × V1=20mg/L × 100ml
V1 =10ml

Hence, 10ml 0f the 200mg/L is measured and diluted to 100ml with methanol. It was then put in an amber glass bottle to prevent photodecomposition until it was ready for use.

2.5.3 Preparation of ascorbic acid

0.01 g of ascorbic acid was weighed and 20 ml of distilled water was used to dissolve it to obtain 500 µg/ml. 2 ml of ascorbic acid solution was taken and distilled water added to make 20 ml (50µg/ml). The rest of the concentrations 25, 12.5 and 6.25 µg/ml were prepared by serial dilution.

2.6 Antimicrobial activity testing

Method

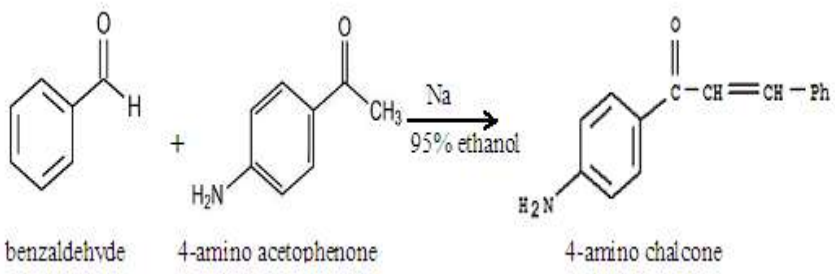
5mg/ml concentration of the plant extracts (leaf, bark and root) and the synthesized chalcone were prepared using ethanol as the solvent. 16 sterile petri dishes were kept under a disinfected screen. 12 test tubes of nutrient agar and 4 test tubes of Sabouraud agar were then melted in bath of boiling water. The melted agar was then stabilized at 45^{o}C for 15 minutes. 1ml each of the test organisms (B. subtilis, E. coli, Staph. aureus, and Candida albicans) was transferred into appropriately labeled petri dishes using a sterile syringe and needle (3 petri dishes for each organism). The stabilized agar was poured into the petri dish containing the suspension of the organism, whirled, covered and then allowed to set. A number 6 cup borer was used to bore 2 holes in the set agar in each of the petri dishes. 1 hole was filled with the extract and the other filled with ethanol as a control. The petri dishes were allowed to stand for 30 minutes before incubation. This was to allow the extracts and ethanol to diffuse into

the agar. The Sabouraud agar was incubated at room temperature for 3days whiles the nutrient agar was incubated at 37^{o}C for 24hours.

2.7 Synthesis of chalcones

1.0612g of benzaldehyde and 1.25ml of the acetophenone were dissolved in 10ml of 95% ethanol in a 25ml conical flask equipped with a magnetic stirring bar. 3.5ml of 6M NaOH solution was then added to the reaction flask. The reaction mixture was stirred for 10 minutes. It was then cooled in an ice-water bath until crystal formation was complete. 2ml of ice-cold water was added to the flask and filtered. The crystals were washed with 5ml of water followed by 5ml of ice-cold ethanol. I was allowed to air-dry. Recrystallization was carried out with 95% ethanol.

The purity of the product was checked by TLC (6:3:1cyclohexane/ethyl acetate/ methanol on silica gel).



2.8 Test for functional groups

2.8.1 Test for Aromatic Hydrocarbons

A small quantity of the synthesized chalcone was placed on a spatula and exposed to direct heat on a Bunsen burner and observed.

2.8.2 Test for Primary Aromatic Amine

5 drops of ethanolic solution of the sample was added to 3ml of dilute Hydrochloric acid. The solution was cooled to below 5°C in crushed ice and a few drops of $NaNO_2$ (made by dissolving 0.1g of $NaNO_2$ in 1ml of water) added.

0.1g of 2-naphthol was dissolved in 1ml of dilute NaOH by warming gently after which 4ml of cold water added. To this solution, the diazonium solution was added. It was then observed for coloured precipitate formation.

3. RESULTS

3.1 Phytochemical screening test.

TEST	LEAVES	BARK	ROOT
Reducing sugars	negative	negative	negative
Anthraquinones	positive	positive	positive
Salkowski test for terpenoids	positive	positive	positive
Dragendorff's test for Alkaloids	positive	positive	positive
Cardiac glycosides	positive	positive	positive
Condensed Tannins	positive	positive	positive
NaOH test for Flavonoids	positive	positive	positive
Lead acetate test for Flavonoids	positive	positive	positive
Ammonia vapour test for Flavonoids	positive	positive	positive
Froth test for Saponins	positive	positive	positive

3.2 THIN LAYER CHROMATOGRAPHY

Solvent system:- ethyl acetate: cyclohexane: methanol (3:6:1).

Sample	Rf	Colour
Chalcone	0.40	Intense yellow
Leaves	0.37	yellow
Root	0.37	yellow
Bark	0.37	Pale yellow

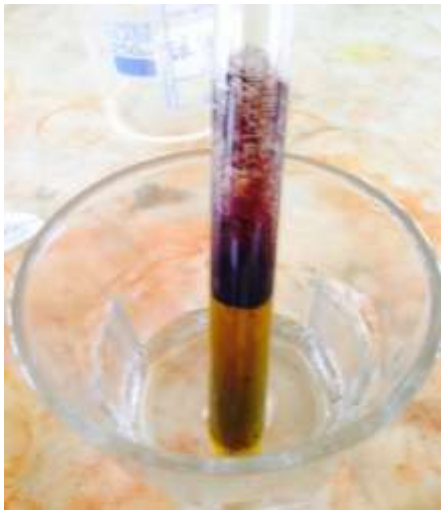
3.3 Functional group test

3.3.1 Test for Aromatic Hydrocarbons

Presence of smoky flame signifying the presence of aromatic ring.

3.3.2 Test for Primary Aromatic Amine

Immediate formation of an intensely coloured precipitate was observed indicating the presence of a primary aromatic amine.

3.4 Antimicrobial Activity Testing.

For *Escherichia coli.*

Sample	Ethanol	Extract+ Ethanol	Extract alone
Leaves	1mm	3mm	2mm
Bark	1mm	2mm	1mm
Root	1mm	3mm	2mm
Chalcone	1mm	7mm	6mm

For *B. subtilis.*

Sample	Ethanol	Extract+ Ethanol	Extract alone
Leaves	3mm	11mm	8mm
Bark	3mm	4mm	1mm
Root	3mm	7mm	4mm
Chalcone	3mm	16mm	13mm

For *Staph. Aureus.*

Sample	Ethanol	Extract+ Ethanol	Extract alone
Leaves	3mm	9mm	6mm
Bark	3mm	4mm	1mm
Root	3mm	7mm	4mm
Chalcone	3mm	10	7mm

For *C. albicans.*

Sample	Ethanol	Extract+ Ethanol	Extract alone
Leaves	5mm	15mm	10mm
Bark	5mm	8mm	3mm
Root	5mm	8mm	3mm
Chalcone	5mm	20mm	15mm

3.5 Anti-oxidant Assay

Ascorbic acid (standard).

Concentration (µg/ml)	Log concentration	%DPPH scavenging act.
250	2.4	69.90
50	1.7	29.00
25	1.4	25.14
12.5	1.1	24.92
6.25	0.8	23.15

Leaves.

Concentration (µg/ml)	Log concentration	%DPPH scavenging act.
500	2.7	65.05
250	2.4	61.52
125	2.1	58.10
62.5	1.8	52.15
31.25	1.5	45.96

Bark.

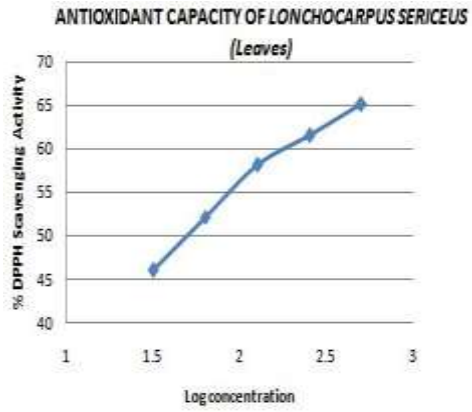
Concentration (µg/ml)	Log concentration	%DPPH scavenging act.
500	2.7	54.58
250	2.4	51.71
125	2.1	41.57
62.5	1.8	36.27

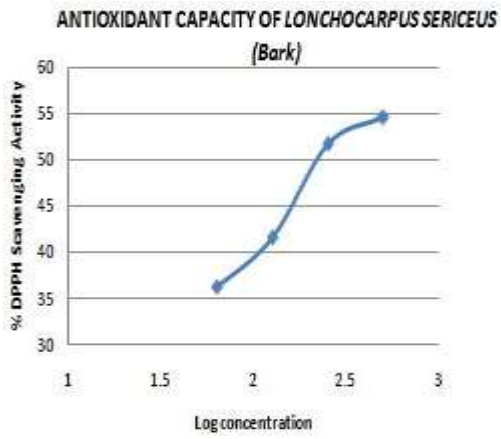
Roots.

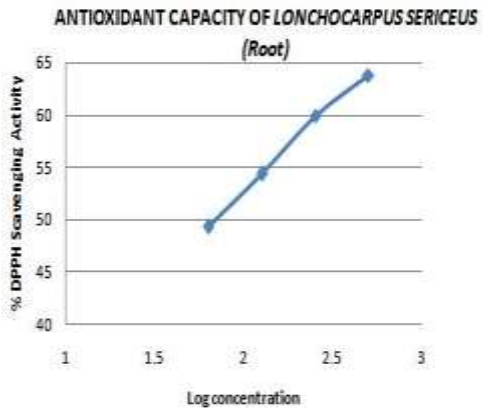
Concentration (µg/ml)	Log concentration	% DPPH scavenging act.
500	2.7	63.73
250	2.4	59.87
125	2.1	54.36
62.5	1.8	49.30

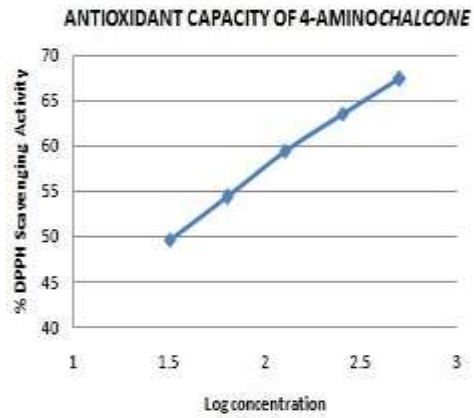
Chalcone.

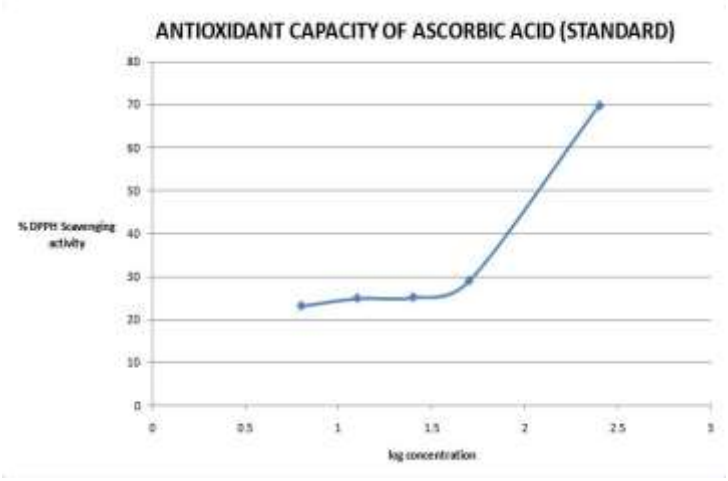
Concentration (µg/ml)	Log concentration	% DPPH scavenging act.
500	2.7	67.36
250	2.4	63.50
125	2.1	59.43
62.5	1.8	54.36
31.25	1.5	49.61











IC50 DETERMINATION

SAMPLE	IC50
Ascorbic Acid	125.89ug/ml
Leaves	50.12ug/ml
Root	70.79ug/ml
Bark	223.87ug/ml
Chalcone	35.48ug/ml

CHAPTER FIVE

4. DISCUSSION

Phytochemical screening of all three organs of the plant, *Lonchocarpus sericeus* (root, bark and leaves) revealed a similarity in the constituents of the parts tested. All the organs gave a positive test for anthraquinones, terpenoids, alkaloids, cardiac glycosides, tannins, flavonoids and saponins. They all also tested negative for reducing sugars. All the plants exhibited potent antioxidant activity. The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. [Polterait O. 1997).

Antibacterial activity obtained in this study varied with organs of plant used. The crude extracts of *L. sericeus* were active against all bacterial strains used and the synthesized chalcones showed maximum zone of inhibition against *E. coli* (6mm), *B. subtilis* (13mm), *Staph. aureus* (7mm), and *Candida albicans* (15mm). Of all the organs of the plants used, the leaves showed highest activity against all the organisms used, followed by the root and then

the bark. This may be due to the presence and quantity of flavonoids (chalcones) and tannins. Tannins are also known for their astringent property and antimicrobial activity [Cowan M, M. 1999]. Ethanol extract of the leaves of *L. sericeus* displayed effective antimicrobial activity against the selected pathogens with the inhibition zone in the range of 2–10 mm with as little concentration as 5mg/ml. It was also observed that all the organs of the plant used had little activity against *E. coli*, which is a gram negative organism. This suggests that the chalcones and other constituents responsible for the antimicrobial activity may not be very active against gram negative organisms as compared to gram positive organisms and fungi.

In the synthesis of chalcones, 4-amino acetophenone and benzaldehyde were used as the reagents whiles sodium hydroxide served as a catalyst. This led to the production of 4-amino chalcone which although is polar, its polarity cannot be compared to that of a polyhydroxy chalcone. The antioxidant activity of flavonoids is related to their structure, especially the hydroxy substitution of the aromatic A- and B-rings and the substitution pattern of the C-ring. According to [Dimitrios T., et al, 2007], the chalcones or flavonoids present in most plants occur in the polyhydroxy form. This may account for why the R_f value of the synthesized chalcone (0.40) differed slightly from that of the three plant samples (0.37). This change in substituent may also have affected the antioxidant and antimicrobial activity since it is known that hydroxy groups are better hydrogen donors than amino groups. As a result, hydroxy groups are better antioxidants. However amino-substituted chalcones have been recorded to have potent antioxidant activity hence very appropriate to have both the amino and hydroxy groups present on the chalcone molecule.

During this study, it was noticed that the synthesized chalcone gave higher antioxidant activity compared with that of the plant samples at all concentrations. This could possibly be related to the quantity of the chalcone present in the plant samples.

The spectrophotometric method of DPPH radicals scavenging has been one of the most commonly used method to estimate the antioxidant activity. [Alasalvar C. et al., 2006] The DPPH test provides information on the reactivity of the test compounds with a stable free radical. The DPPH radical is considered a stable free radical due to the delocalization of electrons available on the entire molecule, showing that the molecules do not dimerise. This electronic delocalization takes place in the radical form, which has violet color observed in the experiments and is responsible for the absorption band at 517 nm, when DPPH was prepared in methanol solution. When the odd electron becomes paired off in the presence of a

free radical scavenger, the absorption reduces and the DPPH solution is decolourized as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract.

When the DPPH radical solution is mixed with a substance that can donate hydrogen atoms, such as polyphenolic compounds, which are good reducing agents because of the hydrogen donor properties of their phenolic hydroxyl groups, obtaining the DPPH reduced form with a resulting decrease in absorbance at 517 nm. Representing the DPPH radical by Z^* and the molecule donates hydrogen atoms as AH, which is responsible for inactivating free radicals formed, the primary reaction would be as follows:

$Z^* + AH = ZH + A^*$

The crude extract from all the organs of *L. sericeus* appeared to be potent as antioxidant. The leaf extract showed higher antioxidant activity compared to the standard Ascorbic acid. After determination of the half maximal inhibitory concentration, IC50 (which is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function) it was found out that the synthesized chalcone has the lowest IC50 followed by the leaf extract, the root extract, the standard (Ascorbic acid) and then the bark having the highest. This suggests that the leaves have a higher quantity of the compound responsible for the antioxidant activity (chalcone) compared to the roots and bark. The lower the IC50, the more potent that extract is. It can also be inferred from the results that the extracts of the leaves and roots are more potent as antioxidants than Ascorbic acid. The bark, however, is less potent than Ascorbic acid, hence more attention can be paid on the use of the roots and leaves of *L. sericeus* as an antioxidant.

One possible explanation of higher activity of the leaf extracts compared to the roots and bark, in the DPPH radicals trapping ability, is the additive or synergic effect that can result from the interaction between polyphenolic constituents present in the fraction with other type of compounds present in the extract, such as essential oils, which could contribute to increase in the antioxidant activity of the extract, because there are reports in the literature about its DPPH radicals scavenging properties.[Pérez R. M. et al., 2006].

5. CONCLUSION

In conclusion, it was found out that all the tested plant plants of *Lonchocarpus sericeus* have both antioxidant and antimicrobial activities and the compound responsible for those

activities of *L. sericeus* is Chalcone, a flavonoid. This therefore suggests that chalcone can be used as a marker for standardizing *Lonchocarpus sericeus.*

The leaves have the highest antioxidant and antimicrobial activity followed by the root and then the bark with the roots and leaves being more potent antioxidants than Ascorbic acid.

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