COMPARATIVE PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *COFFEA ARABICA* LEAF EXTRACTS

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Abstract
Coffea Arabica leaves was analysed for its phytochemical constituents and its antimicrobial activity. Extracts of n-hexane, ethylacetate, acetone, chloroform and methanol respectively were used in the order of increasing polarity. The phytochemical screening revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids, simple sugar (carbohydrates). With the exception of glycosides, all the phytochemicals tested were detected in the sample at varying degrees. Chloroform and methanol extracts of the leaves of coffee arabica were studied for their antibacterial efficacy and antifungal activity. The antimicrobial activities results obtained revealed that the extracts of leaves showed antibacterial activity against Escherichia coli and Staphylococcus aureus except n-hexane. The presence of bioactive compounds and result of antimicrobial activities are an indication that Coffea arabica leaves has medicinal activity.
Keywords: Coffea arabica, phytochemicals, minimum inhibitory concentration (MIC)

Introduction
A traditional medicine is a reliable approach to healthcare delivery in villages and metropolis. The use of plants as medicine to cure illness and to lubricate the wheels of social interaction at interpersonal level is a activity that predates civilization, it is found in every society irrespective of its level of development and sophistication (Odugbemi et al., 2007). Historically, plants have provided a source of inspiration for novel drug compound as plants –derived medicines have made large contributions to human health and well-being (Achan et al., 1980) Mathew (1996) observed that in Nigeria, 70% to80% of the population rely on plants for their primary health care needs. Over 80% of medical care through traditional methods is obtained from medicinal plants while the rest come from other natural product. Sofowora (1993) defined a medicinal plant as any plant, in which one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs. A number of such plants have been used in traditional medicine for many years. Since they seem to work, such plant according to Sofowora (1993), should qualify as medicine plant even when the effectiveness of some of them may not have been proven scientifically. Olowokudejo et al., (2008) explained that the drugs found in today modern society are products of research and developments by major pharmaceutical companies but among the most important materials researched and developed are naturally occurring materials obtained from plant. Phytochemicals are bioactive non-nutrient plant compounds that have protective or disease preventive property. They are formed during the plants normal metabolic processes Okigbo, et al., (2005) they confer plants with odour (terpenoids) pigmentation (tannins and quinines) and flavour (capsacin) Schmelzer and Omino (2003) and are a part of plant natural defense system (Bate-Smith and Swain, 1962). These chemicals are offend referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumerins, glycosides,

gums, polysaccharides, phenols, tannins, saponins, steroids, terpenes and terpenoids. In contrast to synthetic pharmaceuticals based upon single chemicals, many medicinal plants exert their beneficial effects through the additive or synergistic action of several chemical compound acting at single or multiple target sites associated with a physiological process Okigbo, et al., (2005).

Coffee is the most widely consumed beverage in the world and the most commercialized food product. The increase in coffee consumption is due to the information on its health benefits. The compounds of coffee exert antioxidant and other beneficial biological properties. There are nearly 80 identified species of coffee (Clark, 2003). Among them, *Coffeaarabica*accounts for nearly 70% of coffee market and the remaining by*coffeacanephora*(Robusta Coffee) (ICO, 2011 and ABIC, 2011). *Coffeaarabica*is one of the two important classes of coffee belonging to the family of rabiaceae. Coffee has been used traditionally in the treatment of asthma, atropine –poisoning, fever, headache, jaundice, malaria, migraine nareosis, sores and vertigo (Udaya Prakash NK et al., 2014). Caffeine is the most important constituent of coffee which is widely used as stimulant (Coffee, health monograph) A number of beneficial health properties have been attributed to coffee, among them are diuretic, antimicrobial and antioxidant activities (Mohammed and Bayati,(2009). The review of Literature has revealed that most of the work on coffee has been done on the beans of coffee. Thus, in this study, the leaves of *Coffea arabica* was studies to determine the phytochemical constituents and antimicrobial activities.

Materials and methods

Sample collection, identification and preparation. Healthy, uninfected leaves were collected from rural areas of Plateau State and were identified and authenticated at the Department of Biological science Abubakar Tafawa Balewa University, Bauchi. The sample was thoroughly washed with distilled water. The leaves were air dried at room temperature for two weeks after which it was ground into a fine power and stored in air tight container until further use. Soxhlet extraction method was employed in the extraction process. The solvents used in their order of increasing polarity were; n-hexane, ethylacetate, acetone, chloroform and methanol 70 g of pulverized *coffea arabica* leaves was weighed, wrapped properly in a clean whatman filter paper and this was placed in the thimble of soxhlet extractor. 250 ml of n-hexane was poured into round bottom flask of 500 ml capacity placed on the round bottom flask and clamped. Condenser was then fixed on top of soxhlet extractor, and it was connected to a water bath. The heating mantle was switched on and the temperature was adjusted to the boiling point of the solvent in use. The extraction process was continued until a clear liquid was observed indicating completion of extraction. The extract was filtered and poured into a pre-weighed beaker and was fixed on a water bath at 45^{O}C to evaporate to dryness. It was further dried at room temperature for subsequent extraction with other solvents. The above procedure was repeated for ethylacetate, acetone, chloroform and methanol, the weight and percentage recovery of each extract from the respective solvents were calculated and recorded.

Extract preparation

0.5 g (500 mg) of the n-hexane, ethylacetate, acetone, chloroform and methanol extracts were dissolved in 1ml of distilled water to give a concentration of 500 mg/ml each. Two set of nutrient agar wells were prepared for the test and set up. The extracts were then filled into the wells (5) and pre-diffused for 30 minutes. The set up was then incubated at 37^{O}C for 24 hours.

Test organisms

Three different test organisms were selected on the basis of their varying characteristics. They are *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The test organisms were obtained from the Biological Science Department of AbubakarTafawa Balewa Unviersity, Bauchi. The choice of

these Pathogens was based on their incrimination in human disease such as diarrhea, dysentery stomach disorder and skin disease.

Preparation of Mueller Hinton agar and inoculation.

9.5 g of commercially prepared Mueller Hinton agar was weighed and transferred into a flask containing 250 ml of distilled water. The mixture was heated on a hot plate for complete dissolution. It was shakenvigorously while on the plate to avoid charring. It was then sterilized in the autoclave at $121^{O}C$ for (15 minutes) at 151b pressure with the flask plugged with non – absorbent cotton wool, covered with aluminum foil. It was allowed to cool to $40^{O}C$ before plates were poured (Chinoy, 1995). Plates of Mueller Hinton Agar were prepared according to standard method (Ghani 1990).

Sensitivity test method.

The principle of the agar well method is the diffusion of drug (extracts) into the medium that was inoculated previously with the test organism to determine the degree of sensitivity of the organisms against the drug (extract) by the size of the zone of inhibition.

Phytochemical screening.

Preliminary phytochemical screening was carried out on the crude extract to identify the constituents alkanoids terpenoids, steroids, tannins, glycosides, saponins, flavonoids and anthraqunione, using standard method employed by Sofowora(1982); and Trease and Evans (2000).

Alkaloids – Wagner's test

2 cm^3 each of the extract was dissolved in 1 % dilute HCl and filtered the filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown /reddish brown precipitate indicated the presence of alkaloid.

Saponins

To 0.3 g of each of the extracts was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of emulsion. Frothing showed the presence of saponins.

Flavonoids

Two methods were used to test for flavonoids. First, to 2.0 ml of each of aqueous extracts was added 2 ml of 10 % Lead acetate solution. The formation of a yellow precipilate was taken as a positive test for flavonoids.

Terpenoids (Salkowski Test)

3ml of each of the extracts was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration at the interface showed positive result for the pressure of terpenoids.

Steroids.

2ml of acetic anhydride was added to 0.5 g of each of the extracts. 2 ml of H_2SO_4 was also added. A colour change from violet to blue or green indicated the presence of steroids.

Tannins

0.3g of each extract was dissolved in 2.0 ml of distilled water and boiled for two minutes and then filtered . To the filtrate, few drops of ferric chloride solution were added. Blue –black or blue –green precipitates indicate the presence of tannins.

Glycosides

The extracts were hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added . Red precipitate indicated the presence of glucosides.

Anthraquninones (Borntruger's test)

0.5 g of each extract was shaken with 10 ml of benzene and then filtered. About 5 ml of 10 % ammonia solution was added to the filtrate. The mixture was shaken thoroughly. The presence of a pink red or violet colour in the lower phase indicated the presence of free anthraquninones.

Carbohydrate (reducing sugar)

A portion (2 g) of each of the crude extract was dissolved in 5 ml of distilled water. 2 ml of each was placed in a test tube and Fehling's solution (5 ml) was added to each extract and boiled in a water bath for 2 minutes. A brick red colour at the bottom of the test tube indicated the presence of free reducing sugar.

Results.

The leaves of coffea Arabica were extracted sequentially with a soxhlet extractor using five different solvents of varying polarities.

Table 1: Nature and yield of crude extracts of leaves of *Coffea arabica*

Solvent	Colour of extract	Texture	Weight of crude extract (g) W1	Weight of powdered sample (g) W2	Percentage recovery
n-hexane	Dark Green	Oily and sticky liquid	3.4	60.0	5.7
Ethyl acetate	Dark Green	Sticky, thick solid	6.7	53.3	12.6
Acetone	Dark Green	Oily, Thick solid	5.6	54.4	10.3
Chloroform	Light Dark	Dry solid not sticky	3.9	56.1	7.0
Methanol	Dark Green	Very thick sticky, oily solid	3.1	56.9	5.4

The crude extracts were tested for the presence of phytochemicals. The result of the test is presented in table 2 below.

Table 2: phytochemical constituents of the extract of leaves of *Coffea arabica*

Phytochemical	n-hexane	Ethylacetate	Acetone	Chloroform	Methanol
Terpenoids	+	-	-	+	+
Steroids	+	+	-	+	+
Tannins	-	-	-	+	+
Glycosides	-	-	-	-	-
Saponins	-	-	-	-	+
Flavonoids	+	+	+	-	++
Alkaloids	-	-	+	+	++
Anthraquinone	-	+	+	+	+
Reducing sugar	-	-	-	-	+

Key: ++ strong presence; + weakly Presence;- not detected

The crude extracts were tested for activities against some microorganisms and the results of the test is presented in table 3 below

Table 3 The Antimicrobial activity of the crude leaf extracts of *Coffea arabica* on the test organism.

Extracts	Organism/Pathogen	Diameter of zone of inhibition (mm)
n-hexane	*Escherichia coli*	00.00
	Staphylococcus aureus	00.00
	Candida albicans	0.00
Ethylacetate	*Escherichia coli*	10.00
	Staphylococcus aureus	10.00
	Candida albicans	05.00
Acetone	*Escherichia coli*	20.00
	Staphylococcus aureus	15.00
	Candida albicans	00.00
Chloroform	*Escherichia coli*	15.00

	Staphylococcus aureus	10.00
	Candida albicans	10.00
Methanol	*Escherichia coli*	25.00
	Staphylococcus aureus	20.00
	Candida albicans	15.00

Minimum inhibitory concentration [MIC] of the extracts from coffea Arabica leaves was carried out on three extracts Ethyl acetate, acetone and methanol. Results of the test presented in table 4.

Table 4: Minimum inhibitory concentration (MIC) of the extracts from *Coffeaarabica* leaves.

Extracts	200 mg	100 mg	50 mg	25 mg	Pathogen
Ethylacetate	25	12	7	0	*Escherichia coli*
Acetone	13	8	0	0	
Methanol	11	0	0	0	
Ethylacetate	20	9	4	0	*Staphylococcus aerus*
Acetone	15	9	0	0	
Methanol	16	0	0	0	
Ethylacetate	26	15	8	4	*Candidas albican*
Acetone	11	0	0	0	
Methanol	13	11	0	0	

Discussion

From the result obtained in table 1, it could be seen that appreciable percentage recoveries of 5.7, 12.6, 10.3, 7 and 5.4 were obtained using n-hexane, ethyl acetate, acetone, chloroform and methanol respectively. Table 2 revealed the presence of terpenoids, steroids, tannins, glycosides, saponins, flavonoids, alkaloids, anthraquinones and reducing sugar in the leaves of coffea Arabica. Flavonoids were detected in all the extracts except in chloroform extracts. Ojokuku et al., (2010) pointed out that flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, anti-mutagenic, antiviral, antineoplastic, antithrombotic, and vasodilatory activity. It has the ability to scavenge hydroxyl radicals, super oxide, anions lipid peroxy-radical (Okwu and Jasiah 2006).

Saponins was detected only in methanol extract. Saponin causes a reduction of blood cholesterol by preventing its re-absorption (Cashine and Lamb, 2005). Saponins were reported as a major component acting as antifungal secondary metabolite. A wide range of physiological activity of a saponins, steroids, phenols and tannins are found to be more predominant and therefore may be responsible for the antimicrobial action. (Sule WF et al., 2010). They help in controlling cardiovascular disease and in controlling cholesterol in humans (Onwuliri FC 2004).Tannins were detected in methanol and chloroform extracts. They are used in the treatment of wounds emanating from varicose ulcers and hemorrhoids (Nyauyi, 1988: Njoku and Akumufula, 2007). Tannins are used to stop bleeding during circumcision (Joshua, 2006).

Steroid was detected in all the extract except in acetone extract. Steroidal compounds are of importance and interest in pharmacy due to their important role in sex hormones (Okwu, 2001) development and control of body's reproductive system. (Cheng et al., (2002) and Lu et al, (2004) also confirmed the antiviral property of steroids. Steroids are also known to regulate carbohydrate and protein metabolism and posse's anti-inflammatory properties (Cushnie and Lamb, 2005). The presence of terpenoids was detected in methanol, n-hexane and chloroform extracts. Terpenoids form the longest group of plant products and are the

most common ingredient in volatile oils (essential oils). An essential oil is a concentrated, hydrophobic liquid containing volatile aromatic compounds from plants, an oil is essential in the sense that it carries a distinctive scent or essence of the plant. Many essential oils have antiseptic properties (Seenivasanet al., 2006) Terpenoids vary in pharmacological use, as expectorants anti-inflammatory inhibition or cholesterol synthesis, anti-viral and anti-bacterial (Seennivasanet al., 2006).

Alkaloids were detected in all the extracts except in n- hexane and ethyl acetate extracts. Alkaloids have analgesic, anti-inflammatory and adoptogenic activities which help to alleviate pains, develop resistance against disease and endurance against stress (Trease and Evans, 2002). Pure isolated alkanoids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties (Trease and Evans, 2000).

Glycosides stimulate the production of cytokines, they are hormone –like messenger molecules that coordinate the immune defense system, ensuring a smooth and accurate response to foreign invaders. (Alan and Miller, 1996). Glycosides function as anti-cancer agents blocking enzymes that promote tumor growth in the esophagus, breast, lungs, stomach, liver and colon (Tanimure et al, 2005). Cardiac glycosides are used in the treatment of congestive heart failure acting in the heart muscles and increase, renal flow (diuresis) cushnie and lamb, (2005.

Anthraquinone was detected in all the extracts except n-hexane extract. Anthraquinone are naturally occurring phenols found in plants and animals. Phenolic compounds are an important class of compounds due to their ability to function as terminators of free radicals chains or chelators or metal ions. The play an important role in stabilizing the lipid oxidation (Trease and Evans, 2002). Anthraquinone tend to have laxative effects (Axel, 2002).

Table 3 revealed that all the extracts of the leaves exhibited anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus* except n-hexane. Ethyl acetate, chloroform and methanol extracts exhibited anti-fungal activity against *Candida albicans*. Methanol extract exhibited very significant anti-fungal activity against *Candida albicans*. It has been shown that saponins are active anti-fungal agents (Sodipo et al., 1991). The antimicrobial activities of *Coffea arabical* leaves may be due to the presence of these various Phytochemicals (secondary metabolites). The most important bioactive constituents of these plants are alkaloids, tannins, flavonoids, saponins and phenolic compounds (Kuman et al, 2008) Bacteria have different degrees of sensitivity to antimicrobial compounds such as caffeine, volatile non-volatile organic acids and aromatic compounds like phenolic compounds. Caffeine is the most important constituent of coffee which is reported to possess both anti-oxidant and anti-bacterial activity (Naireet al, 2011). Antimicrobial properties of substances are desirable tools in the control or harmful organisms especially in the treatment of infectious disease and in the prevention of food spoilage. The active components usually interfere with the growth metabolism of microorganisms (Aboaba et al., 2001: Mohanta et al., 2007).

The minimum inhibitory activity (MIC) of the extracts of *Coffea arabica* leaves against tested microbes *(Escherichia coli*, *Staphylococcus aureus* and *Candida albican)* ranges from 200 to 25 mg/ml was carried out of *Coffea arabica* against tested microbes ranges from 200 to 100 mg/ml in almost all the extracts and in few instance 50mg/ml tested organisms.

Conclusion

The phytochemical evaluation showed the presence of terpenoids, steroids. Tannis, saponis flavonoids, alkanoids anthraquinone and reducing sugar.

The significance of the leaves of *Coffea arabica* in treatment of disease related to the phytochemical present. The study shows that

the leaves of *Coffea arabica* contain bioactive components known as phytochemicals. Therefore, the therapeutic or medicinal application of the leaves of *Coffea arabica* could be due to the presence of bioactive components.

Recommendations

It is recommended that further work should be carried out on *Coffea Arabica* leaves to determine the following:

i. Anti-oxidant activity of extracts of *coffea arabica* leaves.
ii. Quantify the detected secondary metabolites in the leaves extracts
iii. Carry out a similar study on the stem and root extracts.
iv.

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APPENDICES

Percentage Recovery for leaves Hexane Extract.

Percentage Recovery = $\frac{W1}{W2}$ x 100

$\frac{3.4}{60.0}$x 100

= 5.7%

Percentage Recovery for leaves Ethylacetate Extract.

Percentage Recovery = $\frac{W1}{W2}$ x 100

$\frac{6.7}{53.3}$x 100

= 12.6 %

Percentage Recovery for leaves Acetone Extract.

Percentage Recovery = $\frac{W1}{W2}$ x 100

$\frac{5.6}{54.4}$x 100

= 10.3%

Percentage Recovery for leaves Chloroform Extract.

Percentage Recovery = $\frac{W1}{W2}$ x 100

$\frac{3.9}{56.1}$x 100

= 7.0 %

Percentage Recovery for leaves Methanol Extract.

Percentage Recovery = $\frac{W1}{W2}$ x 100

$\frac{3.1}{56.9}$x 100

= 5.4 %