Phytochemical Screening And Antimicrobial Sensitivity Of Extracts Of The Traditional Medicinal Plant Caloncoba Echinata In Sierra Leone

Lahai Koroma, L. M. Kamara, T.B.R. Yormah, G.M.T. Robert

Abstract: Food tests, Phytochemical Screening and Antimicrobial Sensitivity tests has been carried out on various Plant Organs of the traditional medicinal plant Caloncoba echinata plant in Sierra Leone. The results of the Food Tests indicate presence starch, proteins, glucose and fats/oils in the fruit pulp of **Caloncoba echinata** thus confirming the use of the fruit pulp as a milk substitute for infants in Sierra Leone and as one of the food resources for monkeys and chimpanzees in the Gola Forest. Starch and glucose were absent in the seeds but positive for fats/oils. Phytochemical screening of the petroleum ether, acetone, methanol and Ethanol: Water (50:50) extracts revealed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, terpenoids, tannin and flavonoids by positive reaction with their respective test reagents. Maximum phytoconstituents were found to be present in methanolic, ethanol and ethanol: water (50:50) extracts of the plant. Antibacterial Sensitivity Tests on the various plant organs of **Caloncoba echinata** solvent extracts using Mueller Hilton agar on **Escherichia coli, Salmonella typhimurium, Staphylococcus aureus** and **Bacillus species** indicated that the oil extracted from the seeds and dissolved in Acetone (51%) Xylene (48%), the Oil blank (55%) gave minimum inhibitory activity against **Escherichia coli** and **(37%)** against **Staphylococcus aureus** which are the main bacteria known to stimulate wound and skin infections.This validates the use of the medicinal plant **Caloncoba echinata** as a traditional pharmaceutical

KEY WORDS: Phytochemical screening, Food tests, Antimicrobial sensitivity testing, Soxhlet extraction, Caloncoba echinata, milk substitute and Pharmaceutical.

1 INTRODUCTION

Caloncoba echinata plant is a found in the Gola Forest of the Eastern Province of Sierra Leone and along the West Coast of Africa from Guinea to Western Nigeria. The plant is also found in Brazil and Cuba. It is a traditional medicinal plant used to treat a number of diseases. The plant is a shrub, growing in moist surroundings in primitive forests to a height of 4 to 7 meters. The fruit contains many seeds, 5.5 centimeters in diameter, and is provided with spines It has been reported that the fruit of the plant to comprise of many seeds with very high oil content known as Gorli oil derived from the native tribe Mende name given to the plant in Sierra Leone **[1, 2]**. The Oil is used in the treatment of leprosy and dermal infections **[1, 2 and 3].** In Sierra Leone, the extracts from the various parts of the plant are used as medicines for the treatment of leprosy, cutaneous and subcutaneous parasitic infections (dermal infections or postular eruptions of the skin **[4]**, insecticides and arachnicides, decoction of the leafy twig is used for treatment of diseases in general and also for the treatment of viral diseases such as smallpox, chickenpox and measles, root bark is used in the treatment of menstrual cycle disorders in women, the fruit- pulp is edible and also used as a sweet drink (fruit drink) and as a milk substitute and the oil from the seeds used in hair dressing **[1, 5 and 6]**.

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In Ghana it is called Twi which means elephant's comb. It is also used in the treatment of malaria. Researchers in Denmark have isolated a trio of plant-derived anti-malarial agents from leaves of **Caloncoba echinata [7, 8]**. In Ivory Coast the root extracts of **Caloncoba echinata are** considered to be emmenagogic **[9]**, but the principal application of the plant in all parts of its area of occurrence is in the treatment of leprosy and dermal infections. A decoction of leafy twigs is used to wash sores **[10]** and by enema and in baths for small-pox **[9]**. The pounded seeds are used sometimes with success against lice and mange in the Ivory Coast **[10].** In Liberia, the root, bark and seeds are used as local medicine mostly for treating skin-diseases **[11]**. Compounds reported to be present in **Caloncoba. echinata** are three novel triterpenes, (11R,20R)-11,20-dihydroxy-24-dammaren-3-one (1), (17S,20R,24R)-17,25-dihydroxy-20,24-epoxy-14(18)-malabaricen-3-one (2), and (17R,20S,24R)-17,25-dihydroxy-20,24-epoxy-14(18)-malabaricen-3-one (3), isolated from leaves [12]**,** aleprolic acid **[13],** Four flavonoids **[14]** and dehydroabietinol known to be (11R, 20R)-11, 20-dihydroxy-24-dammaren-3-one and (17S, 20R, 24R)- and (17S, 20S, 24R)-17,25-dihydroxy-20,24-epoxy-14(18)-malabaricen-3-one **[7]**. The present work examines Food tests, phytochemical screening and Antimicrobial sensitivity of various extracts of the plant organs of traditional medicinal plant Caloncoba echinata.

MATERIALS AND METHODS

Collection and preparation of dried plant materials

Fresh plant materials Caloncoba echinata were collected from the Gola Forest and dried under the shade and not the sun so as to protect the thermo labile components if present from being chemically transformed. It was then reduced in size by crushing it into smaller pieces using the hand. After the plant material had been dried, it was each grounded using a laboratory mill and kept in a proper container until the time of the extraction. The plants organs investigated are Leaves (L), Stem bark (SB), Root bark (RB) and the Fruit (F) of Caloncoba

echinata. A voucher specimen of each the plant parts of **Caloncoba echinata** was deposited in the Herbarium of the Botany Department, Fourah Bay College (University of Sierra Leone). The plant materials are used to carry out the following analyses described below:

Food tests on Fruit pulp and seed
Soxhlet extraction of powdered plant materials
Phytochemical screening (powdered plant materials)
Antimicrobial Sensitivity testing (Solvent extracts of the powdered plant materials)

EXPERIMENTAL

Soxhlet extraction

Soxhlet extraction was carried out on each of the powdered plant materials using solvents of increasing polarity (i.e. petroleum ether [60-80^{o}C], acetone, methanol, 95% ethanol and water: ethanol [50:50] at a temperature of 70˚C. Each of the solvent extracts was concentrated, reduced to a semisolid mass using a Rotary Evaporator at 50^{o}C and then dried using a water bath at 50˚C. 10mg of each of the dried extract was placed in a labeled specimen bottle and 10ml of the required solvent added to dissolve the extracts and stored in a refrigerator for antimicrobial sensitivity testing.

Phytochemical screening

The Phytochemical screening involved testing each of the **Caloncoba echinata solvent extracts** (**CESE**) for their content of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of bioactive compounds such as alkaloids, tannins, flavonoids, steroids/terpenes and saponins present in each of the solvent extracts of the various plant parts investigated [15, 16, 17, 18 19 and 20]. Food tests on the fruit pulp and seeds of the plant were also carried out.

Antimicrobial Sensitivity test

The following bacteria isolates were supplied by the Department of Microbiology, Connaught Hospital, and Freetown;

i. Escherichia coli
ii. Salmonella enterica – Gram negative
iii. Staphylococcus aureus
iv. Bacillus anthracis (Bacillus sp.) – Gram positive

Antimicrobial analysis was carried out on the extracts of the various plant organs using the disc diffusion method on the test organisms; Escherichia coli, Salmonella enterica, Staphylococcus aureus and Bacillus anthracis (Bacillus sp.) with Muller-Hinton agar as culture medium. The minimum inhibitory zones were determined in percentage efficacy relative to the standard antibiotics Ciprofloxacin and the most active extract in parallel experiments in order to control the sensitivity of the test microorganisms **[21].** All tests were performed in duplicate.

RESULTS AND DISCUSSIONS

The results of the Food tests and Phytochemical screening of the various plant organs are reported and discussed in Tables **1-2.**

Table 1: *Tests for carbohydrates and reducing sugar, proteins and fats and oils in the various plant organs of Caloncoba echinata*

Table 1 here
KEY: 1. Iodine Test, 2. Biuret test, 3. Xanthoproteic test, 4. Benedict's test, 5. Fehling's Test, 6. Barfoed's Test, 7. Molisch's test, 8. Translucent test**, 9. Sudan III test: + + + = Intense; + + = Moderate; + = Slight; - = Absent L = Powdered leaves, SB = Powdered stem bark, RB = Powdered root bark**

Table 1 indicates the presence starch, proteins, glucose and fats/oils are present in the fruit pulp of **Caloncoba echinata**. Traditionally, the fruit pulp is used as a milk substitute for infants. The above food test results confirmed the use of fruit pulp as a milk substitute. It also confirmed the use of the fruit by monkeys and chimpanzees as one of their food resources in the Gola Forest. It is also another source of producing alcohol from natural plant source by fermenting the fruit pulp. Further work on the fruit pulp is recommended in order to provide an alternative food substitute for children. Starch and glucose were absent in the seeds but tested positive proteins and fats/oils.The test results shown in **Table 1** also indicate the presence of carbohydrates and reducing sugar in the various solvent extracts of Caloncoba echinata with the exception of the petroleum ether extracts. Food tests on the other plant organs indicate the presence of Amino acids and Proteins in the acetone, methanol, ethanol and Ethanol : Water (50:50) extracts of **Caloncoba echinata**. The petroleum ether extracts gave negative test.

TABLE 2: *Tests for Alkaloids, Tannins, Flavonoids, triterpenes and saponins in the various solvent extracts of Caloncoba echinata*

Table 2 here
KEY: 1. Mayer's test, 2. Hager's test, 3. Wagner's test, 4. Dragendroff's test, 5. Ferric chloride test, 6. Gelatin test, 7. Iodine test, 8. Nitric acid test, 9. Shinoda's test, 10. Alkaline reagent test, 11. Sodium hydroxide test: 12. Libermann-Burchard test, 13. Salkowski's test, 14. Keller kelliani test, 15. Borntrager's test, 16. Froth test: + + + = Intense; + + = Moderate; + = Slight; - = Absent L = Powdered leaves, SB = Powdered stem bark, RB = Powdered root bark

With the exception of the petroleum ether and acetone extracts, all the other solvent extracts of the plant organs investigated gave positive tests for Alkaloids, tannins and phenolic compounds and glycosides as shown in **Table 2**. Flavonoids and triterpenes are present in the various solvent extracts of **Caloncoba echinata** with exception of the Petroleum Ether Extracts.

Summary of the results of the phytochemical screening of the various solvent extracts.

Phytochemical screening of petroleum ether, acetone, methanol and Ethanol: Water (50:50) extracts revealed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, terpenoids, tannin and flavonoids by positive reaction with their respective test reagents. The results indicate maximum presence of phytoconstituents in methanolic, ethanol and ethanol: water (50:50) extracts of the plant The curative properties of medicinal plants are due to the presence of various secondary metabolites such as alkaloids,

flavonoids, glycosides, phenols, saponins, triterpenes/sterols etc. **[22**] The tests on the successive extracts of **Caloncoba echinata** revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, triterpenes and tannins, which validates the use of the various plant organs of the plant **Caloncoba echinata** as a traditional pharmaceutical. Hence these preliminary screening tests are useful in the detection of the bioactive principles which subsequently leads to drug discovery and development.

Results and discussions on antibacterial sensitivity testing of plant solvent extracts.

The results of Antibacterial sensitivity tests carried out on the various **Caloncoba echinata** solvent extracts using Mueller Hilton agar on **Escherichia coli, Salmonella typhimurium, Staphylococcus aureus** and **Bacillus species** are shown in **Tables 3 – 4**

Table 3: *Antibacterial activity of oil extracted from the seed in various selected solvents*

TABLE 3 here

KEY: [a] = Values are zones of inhibition diameter (mm) **Ec** =Escherichia coli, **St** = Salmonella typhimurium,
Sa = Staphylococcus aureus, **B** =Bacillus sp - = Concentration not sensitive to Test organism, **Oil blank,** % = Percentage efficacy relative to the standard drug Ciprofloxacin, **DEZ** – Diethyl ether, **A** – Acetone,
DCM – Dichloromethane, **PZ** – Petroleum ether, **X** = Xylene, **L** – Leaves, **SB** – Stem bark, **RB** – Root bark.

The results in **Table 3** indicate that the oil extracted from the seeds and dissolved in Acetone (**51%),** Xylene (**48%**), the Oil blank (**55%**) gave minimum inhibitory activity against **Escherichia coli** and **37%** against **Staphylococcus aureus.** This indicates that the oil extracted from the seeds of **Caloncoba echinata** is more effective in its crude form than when dissolved in various other solvents. The oil can therefore be used as an additive to skin formulations in cosmetic productions.

Table 4: *Antibacterial sensitivity tests on the various solvent extracts of the plant organs of Caloncoba echinata*

Table 4 here

KEY: [a] = Values are zones of inhibition diameter (mm) **Ec** =Escherichia coli, **St** = Salmonella typhimurium, **Sa** = Staphylococcus aureus, **B** =Bacillus sp - = Concentration not sensitive to Test organism % = Percentage efficacy relative to the standard drug Ciprofloxacin, **EW** – Ethanol: Water (50:50), **L** – Leaves, **SB** – Stem bark, **RB** – Root bark. **Table 4** indicates that the Petroleum ether and Acetone extracts of each of the plant organs investigated had no antibacterial activity against the test organisms used. The Methanol extract how ever showed significant antimicrobial activity against the test organisms used with the leaf extract **45%** for **Escherichia coli**, **53%** for **Salmonella typhimurium**. The stem bark gave **44%** while the root bark gave **62%** for **Salmonella typhimurium**. Ethanol extract showed moderate antimicrobial activity against the test organisms used with the leaf extract **44%** for **Salmonella typhimurium**, stem bark gave **56%** while the root bark gave **24%** for **Salmonella typhimurium** and **38%** for **Escherichia coli** and the Ethanol: Water (**50:50**) solvent mixture gave moderate antimicrobial activity against the test organisms used with the leaf extract of **47%** for **Salmonella typhimurium**, stem bark gave **32%** while the root bark gave **44%** for **Salmonella typhimurium** and **41%** for **Escherichia coli**.

Summary of the results of antibacterial sensitivity testing of plant extracts investigated.

Antibacterial Sensitivity Tests on the various plant parts of **Caloncoba echinata** solvent extracts using Mueller Hilton agar on **Escherichia coli, Salmonella typhimurium, Staphylococcus aureus** and **Bacillus species** has been investigated. The results indicate that the oil extracted from the seeds and dissolved in Acetone (51%) Xylene (48%), the Oil blank (55%) gave minimum inhibitory activity against **Escherichia coli** and **(37%)** against **Staphylococcus aureus** which are the main bacteria known to stimulate wound and skin infections. This confirmed the use of the oil extracted from the seed as medicines for the treatment of leprosy, cutaneous and subcutaneous parasitic infections (dermal infections) **[17, 18, 19 and 20]** Petroleum ether and Acetone extract of each of the plant parts gave no antimicrobial activity against the test organisms used. The methanol extract of each of the plant parts showed significant antimicrobial activity against the test organisms used with the leaf extract of **45%** for **Escherichia coli**, **53%** for **Salmonella typhimurium**. The stem bark gave **44%** while the root bark gave **62%** for **Salmonella typhimurium**. Also, the ethanol extract of each of the plant parts showed moderate antimicrobial activity against the test organisms used with the leaf extract of **44%** for **Salmonella typhimurium**. The stem bark gave **56%** while the root bark gave **24%** for **Salmonella typhimurium** and **38%** for **Escherichia coli**. The Ethanol: Water (50:50) solvent extracts of each of the plant parts showed moderate antimicrobial activity against the test organisms used with the leaf extract of **47%** for **Salmonella typhimurium**. The stem bark gave **32%** while the root bark gave **44%** for **Salmonella typhimurium** and **41%** for **Escherichia coli** thus confirming the use of the decoction of the leafy twig and root bark for treatment of diseases in general, viral diseases such as smallpox, chickenpox and measles and menstrual cycle disorders in women.

CONCLUSION

Phytochemical screening of petroleum ether, acetone, methanol and Ethanol: Water (50:50) extracts revealed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, terpenoids, tannin and flavonoids in the various plant organs investigated. The screening gave maximum Phytoconstituents in methanolic, ethanol and ethanol: water (50:50) extracts. Alkaloids, Flavonoids, saponins, Sterols and Tannins are some of the identified secondary plant metabolites known to have pharmacologically active compounds **[23, 24].** They play significant role in both traditional and modern medicines. Flavonoids have been reported to prevent the defoliation and loss of specialized function of the cells of a tissue or organ, destroy fats and fibrous tissues in living organisms and prevent human degenerative diseases [25**],** anti-allergic [26], Anti-inflammatory **[26, 27],** antioxidant **[27],** anti-microbial (antibacterial) **[28, 29]** antifungal an antiviral **[30, 31]]**, anti-cancer [27, 32] and anti-diarrhoeal activities [33]**. Saponins [34]** cause abortion especially Yamogenin which is widely used as starting material in the synthesis for birth control pills. Hence the presence of alkaloids, flavonoids, cardiac glycosides, saponins, triterpenes and tannins in various plant organs of **Caloncoba echinata** plant support the

use of the medicinal plant Caloncoba echinata as a traditional pharmaceutical. Antibacterial Sensitivity Tests on the various plant organs of **Caloncoba echinata** solvent extracts using Mueller Hilton agar on **Escherichia coli, Salmonella typhimurium, Staphylococcus aureus** and **Bacillus species** has been investigated. The results indicate that the oil extracted from the seeds and dissolved in Acetone 51%) Xylene (48%) and the Oil blank (55%) gave minimum inhibitory activity against **Escherichia coli** and **(37%)** against **Staphylococcus aureus** which are the main bacteria known to stimulate wound and skin infections. This confirmed the use of the oil extracted from the seed as medicines for the treatment of leprosy, cutaneous and subcutaneous parasitic infections (dermal infections)**.** Petroleum ether and Acetone extract of each of the plant parts gave no antimicrobial activity against the test organisms used. The methanol extract of each of the plant parts showed significant antimicrobial activity against the test organisms used with the leaf extract of **45%** for **Escherichia coli**, **53%** for **Salmonella typhimurium**. The stem bark gave **44%** while the root bark gave **62%** for **Salmonella typhimurium**. Also, the ethanol extract of each of the plant parts showed moderate antimicrobial activity against the test organisms used with the leaf extract of **43%** for **Salmonella typhimurium**. The stem bark gave **56%** while the root bark gave **24%** for **Salmonella typhimurium** and **39%** for **Escherichia coli**. The Ethanol: Water (**50:50**) solvent extracts of each of the plant parts showed moderate antimicrobial activity against the test organisms used with the leaf extract of **47%** for **Salmonella typhimurium**. The stem bark gave **32%** while the root bark gave **44%** for **Salmonella** t**yphimurium** and **41%** for **Escherichia coli** thus confirming the use of the decoction of the leafy twig and root bark for treatment of diseases in general, viral diseases such as smallpox, chickenpox and measles and menstrual cycle disorders in women.

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Table 1: *Tests for carbohydrates and reducing sugar, proteins and fats and oils in the various plant organs of Caloncoba echinata*

Solvent Extracts		Test Reagents								
		1	2	3	4	5	6	7	8	9
Petroleum ether	L	-	-	-	-	-	-	-	-	-
	SB	-	-	-	-	-	-	-	-	-
	RB	-	-	-	-	-	-	-	-	-
Acetone	L	-	+	+	+	+	+	+	-	+
	SB	-	++	++	++	++	++	++	-	++
	RB	-	+	+	+	+	+	+	-	+
Methanol	L	+++	-	-	+++	+++	+++	+++	-	++
	SB	+	-	-	++	++	++	++	-	++
	RB	+	-	-	+	+	+	+	-	+
Ethanol	L	+++	+	++	+++	+++	+++	+++	-	++
	SB	+	+	++	++	++	++	++	-	++
	RB	+	+	+	+	+	+	+	-	+
Ethanol :Water (50 : 50)	L	+++	+	+	+++	+++	+++	+++	-	++
	SB	+	+	+	++	++	++	++	-	++
	RB	+	+	++	+	+	+	+	-	+
Fruit Pulp		++	+++	++	+++	+++	++	+	+	+
Seeds		-	+++	-	-	-	-	-	+++	+++

TABLE 2: *Tests for Alkaloids, Tannins, Flavonoids, triterpenes and saponins in the various solvent extracts of Caloncoba echinata*

Solvent Extracts		Test Reagents															
		Alkaloids				Tannins				Flavonoids			triterpenes		Saponins		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Petroleum ether	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	SB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	RB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	L	-	-	-	-	-	-	-	-	+	+	++	+	+	-	-	++
	SB	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	++
	RB	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
Methanol	L	++	++	+++	+	+++	++	++	++	+++	+++	++	++	++	+	+	+++
	SB	++	++	+++	+	++	++	+	++	++	++	++	++	++	+	+	+++
	RB	++	++	+++	+	++	++	+	++	+	+	++	++	++	+	+	+++
Ethanol	L	++	++	+++	+	++	++	+	++	+++	+++	+++	++	++	+	+	+++
	SB	++	++	+++	+	+	++	+	++	++	++	++	++	++	+	+	+++
	RB	++	++	+++	+	+	++	+	++	+	+	+	++	++	+	+	+++
Ethanol:Water (50 : 50)	L	++	++	++	+	++	++	+	++	+++	+++	+++	+++	+++	+	+	+++
	SB	++	++	++	+	++	++	+	++	++	+	+	+++	+++	+	+	+++
	RB	++	++	++	+	++	++	+	++	+	+	+	+++	+++	+	+	+++

Table 3: *Antibacterial activity of oil extracted from the seed in various selected solvents*

No.	Plant sample	Bacteria [a]			
		Ec (%)	St (%)	Sa (%)	B (%)
1	001-DEZ	-	-	-	-
2	002-A	15(51)	-	-	-
3	003-DCM	-	-	-	-
4	004-PZ	-	-	-	-
5	005-X	14(48)	-	-	-
6	000 Oil blank	16(55)	-	11(37)	-
7	Ciprofloxacin	29(100)	34(100)	30(100)	28(100)

Table 4: *Antibacterial sensitivity tests on the various solvent extracts of the plant organs of Caloncoba echinata*

Solvent	Plant Organ	Bacterial isolates			
		EC (%)	St(%)	Sa(%)	B(%)
Petroleum ether	L	-	-	-	-
	SB	-	-	-	-
	RB	-	-	-	-
	Solvent Blank	-	-	-	-
Acetone	L	-	-	-	-
	SB	-	-	-	-
	RB	-	-	-	-
	Solvent Blank	-	-	-	-
Methanol	L	13(45)	18(53)	-	-
	SB	-	15(44)	-	-
	RB	-	21(62)	-	-
	Solvent Blank	-	-	-	-
Ethanol	L	-	15(44)	-	-
	SB	-	19(56)	-	-
	RB	11(38)	8(24)	-	-
	Solvent Blank	-	-	-	-
Ethanol:Water (50:50)	L	-	16(47)	-	-
	SB	-	11(32)	-	-
	RB	12(41)	15(44)	-	-
	Solvent Blank	-	-	-	-
Ciprofloxacin		29(100)	34(100)	30(100)	28(100)