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Detection and Distribution of Some Phytochemicals in *Theobroma Cacao* Tree

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Abstract

Theobroma cacao tree parts (leave, stem bark, root bark, pulp, bean, and bean coat) were dried and grinded to fine particle size of 75 µm mesh size. Each of these samples was dissolved in five different solvents (aqueous, methanol, ethanoic acid, petroleum ether and ethanol). The extract obtained was phytochemicals screened using standard procedure for the presence of flavonoids, tannins, alkaloids and saponins. The results revealed the presence of the phytochemicals in the order alkaloids>tannins>flavonoids> saponins. The distribution was stem bark>pulp;root>bean; root bark; leaves>bean coat for alkaloids, tannins, flavanoids and saponins respectively.

In conclusion, *Theobroma cacao* parts (leaf, bean bean coat, bean, pulp, root and stem barks) and other agricultural run-off that are mostly under-utilized and discarded as wastes can be found useful and applicable in pharmaceutical industry and may bring high reduction on expenditure on the purchase of related compounds used in pharmacognosy from foreign lands and also help in waste management and control.

Keywords: *Theobroma cacao* ; Solvents; Phytochemicals; Presence; Distribution

Introduction

The therapeutic properties of plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, flavonoids, saponins, resin, volatile gums, anthraquinones and cardiac glycosides in the plants [1-2]. The potential values of these bioactive agents in food and pharmaceutical industries as preservatives and antimicrobial agents had been reported by Ajiboso.

Several Scientists have proved the potency and efficacy of some of these bioactive agents in some indicator species such as bacteria, fungi and virus [3]. According to Joseph and Hang (2005), tea-Camellia sinensis phenolic compounds possess antioxidant properties that may lower the risk of some cancers and coronary heart disease; anti-HIV properties of flavonoid compounds of the tea were also reported by Okogon. Anti-mycotic properties of saponins and anti-bacterial properties of flavonoids and tannins have also been documented. Class of alkaloids was regarded to be among the highest poisons known [4].

In view of the enormous values of some of these bioactive agents such as flavonoids, alkaloids, tannins and saponins, every plant is a potential constituent and source of these important phytochemicals that play important roles in drug discovery and development.

Cocoa is an economic important fruit and an important cash crop in Nigeria, the fruit can be consumed or traded at both local and international market or may be stored as seedling for planting. The other parts such as leave, stem and root are mostly under-utilized and discarded away as wastes.

It is therefore the interest of this present study to determine the phytochemical constituents of cocoa tree leaf, stem bark, root bark, bean, and pod, bean coat for the presence of some phytochemicals.

Materials and Methods

Sample collection

Fresh ripe cocoa pods of matured cocoa tree (*Theobroma cacao*), the stem and root barks were collected from a cocoa plantation at Ile-Oluji, Ondo-State, Nigeria in November 2020.

Preparation of plant sample

Plant samples were prepared according to the procedure described by Ajiboso [5]. Fresh samples were sun dried for 3 days followed by grinding into fine particle size of 75 µm sieve range using sterile Electric blender (HR 2815, Phillips Co. Holland). 100 g each of the grinded particles was weighed with the aid of a Triple beam balance (USA, patent No. 2,729,439). The weighed grinded particles were stored at room temperature in clean sterile bottles (Simax, Czechoslovakia).

Preparation of the extracts

100 ml of each solvent (distilled water, methanol, ethanoic acid, 95% ethanol and petroleum ether was added to 20 g of each plant sample in different beakers (Simax, Czechoslovakia). Each of these beakers was tightly covered with cork and vigorously shaken to ensure that there was even dispersion of the particles in the solvents. It was allowed to stand for 24 hours for adequate pigment extraction before it was filtered using

Whatman No.1 filter paper. The filtrates (extracts) were stored in sterile bottles for an hour before phytochemical analysis.

Chemicals and reagents

All chemicals and reagents used were of analytical grade (Poole-England). The reagents used were prepared with distilled water unless otherwise stated.

Phytochemical analysis

Phytochemical analysis was done according to the procedures described by Harbone (1998) on the extracts. The extracts were analyzed for the presence of tannins, flavonoids, alkaloids, and saponins.

Tannins: 2 drops of 5% $FeCl_3$ was added to 1 ml of the extracts. A greenish precipitate observed in each extract tested indicated the presence of tannins.

Flavonoids: 1 ml 10% NaOH was added to 3 ml of the extract. A creamy precipitate observed in each extract tested indicated the presence of Flavonoids.

Alkaloids: 2 drops of Mayer's reagent was added to 1 ml of the extracts. A creamy precipitate observed in each extract tested indicated the presence of alkaloids.

Saponins: Frothing test: 2 cm^3 of the extracts in a test tube was vigorously shaken for 2 minutes. Frothing observed in each extract tested indicated the presence of saponins.

Results and Discussion

The results of the phytochemical analysis of cocoa tree are presented in Table 1 to 5. The results revealed excess flavonoids in ethanoic acid extracts of bean, pod, leaf and methanol extract of pod; trace levels in ethanoic acid extracts of root, stem bark and bean coat while the root bark extracts and extracts of stem bark and bean coat showed the absence of lavonoid compounds.

Alkaloids were excess in the ethanoic acid extract of pod; aqueous and ethanol extracts of root bark, leaf, pod and methanol extracts of the stem bark, bean, bean coat, pod and petroleum ether extracts of the stem bark. Ethanoic acid extracts of root bark, stem bark, bean, bean coat and leaf showed trace levels of alkaloids while few extract mostly petroleum ether extracts revealed the absence of alkaloids.

From the results obtained, the level of tannins were detected excess in ethanol and methanol extracts of root bark, stem and aqueous extracts of stem bark, pod and ethanol extract of pod. Tannins were trace in petroleum ether extracts of root bark and stem bark; aqueous and ethanol extracts of bean, bean coat and methanol extract of bean coat. Tannins were observed absent in the leaf and ethanoic acid extracts, petroleum ether extracts of bean, bean coat and pod, aqueous extracts of root bark, stem, bean, bean coat, pod and ethanol extracts of stem bark and bean.

Petroleum ether extract of stem bark showed the presence of saponins in excess; saponins were trace in ethanol extracts of bean, bean coat and leaf; methanol extracts of root bark and aqueous extracts of root, stem bark, leave and pod. Stem extracts and most of the extracts did not show the presence of saponins.

Table 1: Showing the presence and distribution of phytochemicals in *Theobroma cacao* root bark extracts.

E/PS	RBE			
	F	A	T	S
AQ	-	++	+	-
ME	-	+	+	+
ETA	-	+	-	+
PE	-	+	+	-
ET	-	++	++	-

++ present much; + present; - absent. ++ present much; + present; - absent.

KEYS

E/PS=Extracts/Plant Samples

F=Flavonoids, A=Alkaloids, T=Tannins, S=Saponins

AQ=Aqueous; ME=Methanol; ETA=Ethanoic acid; PE=Petroleum ether; ET=Ethanol

RBE=Root Bark Extract; SBE=Stem Bark Extract; BE=Bean Extract; BCE=Bean Coat Extract; LE=Leave Extract; PE=Pod Extract

Table 2: Showing the presence and distribution of phytochemicals in *Theobroma cacao* stem bark extract.

E/PS	SBE			
	F	**A**	**T**	**S**
AQ	-	+	++	+
ME	-	++	++	-
ETA	+	+	-	-
PE	-	++	+	++
ET	-	++	+	-

++ present much; + present; - absent. ++ present much; + present; - absent.

KEYS

E/PS= Extracts/Plant Samples

F=Flavonoids, A=Alkaloids, T=Tannins, S=Saponins

AQ=Aqueous; ME=Methanol; ETA=Ethanoic acid; PE=Petroleum ether; ET=Ethanol

RBE=Root Bark Extract; SBE=Stem Bark Extract; BE=Bean Extract; BCE=Bean Coat Extract; LE=Leave Extract; PE=Pod Extract

Table 3: Showing the presence and distribution of phytochemicals in *Theobroma cacao* bean extract.

E/PS	BE			
	F	**A**	**T**	**S**
AQ	+	-	+	-
ME	-	++	-	-
ETA	++	+	-	-
PE	-	+	-	-
ET	-	-	+	+

++ present much; + present; - absent. ++ present much; + present; - absent.

KEYS

E/PS=Extracts/Plant Samples

F=Flavonoids, A=Alkaloids, T=Tannins, S=Saponins

AQ=Aqueous; ME=Methanol; ETA=Ethanoic acid; PE=Petroleum ether; ET=Ethanol

RBE=Root Bark Extract; SBE=Stem Bark Extract; BE=Bean Extract; BCE=Bean Coat Extract; LE=Leave Extract; PE=Pod Extract.

Table 4: Showing the presence and distribution of phytochemicals in *Theobroma cacao* leaf extract.

E/PS	LE			
	F	**A**	**T**	**S**
AQ	-	++	-	+
ME	-	+	-	-
ETA	++	+	-	-
PE	+	-	-	-
ET	+	++	-	+

++ present much; + present; - absent. ++ present much; + present; - absent.

KEYS

E/PS=Extracts/Plant Samples

F=Flavonoids, A=Alkaloids, T=Tannins, S=Saponins

AQ=Aqueous; ME=Methanol; ETA=Ethanoic acid; PE=Petroleum ether; ET=Ethanol

RBE=Root Bark Extract; SBE=Stem Bark Extract; BE=Bean Extract; BCE=Bean Coat Extract; LE=Leave Extract; PE=Pod Extract.

Table 5: Showing the presence and distribution of phytochemicals in *Theobroma cacao* pod extract.

E/PS	PE			
	F	A	T	S
AQ	+	++	++	+
ME	++	++	+	-
ETA	++	++	-	-
PE	-	-	-	-
ET	-	++	++	-

++ present much; + present; - absent. ++ present much; + present; - absent.

KEYS

E/PS=Extracts/Plant Samples

F=Flavonoids, A=Alkaloids, T=Tannins, S=Saponins

AQ=Aqueous; ME=Methanol; ETA=Ethanoic acid; PE= Petroleum ether; ET= Ethanol

RBE= Root Bark Extract; SBE=Stem Bark Extract; BE=Bean Extract; BCE=Bean Coat Extract; LE=Leave Extract; PE=Pod Extract

Alkaloids and tannins were found to be more abundant than flavonoids and saponins in cocoa tree. Class of alkaloids has been employed as muscle stimulant, narcotic and psychotropic drugs [4]. The antimicrobial activity of alkaloids against flavonoids and tannins resistant microorganisms had also been documented [5-6]. Report had been made on the high level of tannins in the bean coats of dry beans than the bean.

This report agrees with the observed level of tannins in bean coat and bean in this present study. Germination, soaking, cooking and the discarding of the broth (cook-water) remove 50%, 38% and 77% tannin content of beans respectively [4]. Tannins were excess in other plant parts except bean, bean coat and the leaves. It may be possible for the tannins content of bean and bean coat to be naturally high, but the trace levels detected in bean and bean coat may be due to soaking of the beans prior to germination and post-harvest processing of crops such as fermentation and also through bean coat removal by either dehulling or milling. Saponins were observed to be present in low level and virtually non-existent in all the extracts of the stem. The pulp, stem and root also contained flavonoids, alkaloids and tannins in excess. Antimicrobial activities of tannins due to the precipitation of nutritional protein have also been documented [3-5].

The presence of phytochemicals in excess in stem bark of cocoa tree suggests stem bark as of the major sites of concentration of most phytochemicals since stem bark predominates in herbs for concoction preparation in trado-medical practices.

The detection and distribution of phytochemicals in cocoa tree are in the order alkaloids>tannins>flavonoids>saponins and stem bark>pulp; root>bean; root bark; leaves>bean coat. One important observation made in this present study is that the extraction of these phytochemicals depends on plant part and solvent used.

Maximum extraction of flavonoids from pulp, bean and leaf was achieved through the use of methanol and ethanoic acid. The solvents ethanol, methanol and aqueous observed to be the most suitable solvents for maximum extraction of tannins and alkaloids while the extraction of saponins content of cocoa tree can be achieved with petroleum ether.

The potency of organic solvents in the extraction of phytochemicals from the plant than water in this present study is in accordance with observations made in earlier studies of Ademoroti [7,8].

Conclusion

From the work carried out, *Theobroma cacao* can be seen as a good source of these important phytochemicals. Its promising potential in its adaptive uses as food, source of foreign exchange earnings coupled with its phytochemical constituents show the importance of cocoa tree in the economy of Nigeria. Conclusively, *Theobroma cacao* parts (leaf, bean coat, bean, pulp, root and stem barks) and other agricultural run-off that are mostly under-utilized and discarded as wastes can be found useful and applicable in pharmaceutical industry and may bring high reduction on expenditure on the purchase of related compounds used in phamacognosy from foreign lands and also help in waste management and control [9-11].

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