

Comparison of Efficiency of Different Solvents used for the Extraction of Phytochemicals from the Leaf, Seed and Stem Bark of *Calotropis Procera*

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Abstract: Phytochemicals screening were carried out on different solvent extracts of leaf, seed and stem bark of *Calotropis procera* to compare the efficiency of different solvents which are Ethanol, Diethyl Ether, Chloroform, Methanol, N-hexane, De ionised water, Ethyl Acetate, Acetone, Petroleum Ether and Isopropanol. Qualitative analyses of twelve secondary metabolites (alkaloid, saponin, flavonoid, terpenoid, glycosides, tannin, phenolic compounds, carbohydrate, amino acid, quinines, oxalate and sterol) were carried out. The results showed that eleven (alkaloid, saponin, flavonoid, terpenoid, glycosides, tannin, phenolic compounds, carbohydrate, amino acid, quinines and oxalate) of the twelve analyzed were present in stem bark, ten phytochemicals (alkaloid, saponin, flavonoid, terpenoid, glycosides, phenolic compounds, carbohydrate, amino acid, quinines and sterol) were present in the seed while all the twelve metabolites were present in the leaf. Generally, water proved to be most effective as it was able to extract numbers of phytochemicals. The presence of various phytochemical further established the previous findings that parts of *calotropis procera* are good sources of therapeutical compounds.

Keywords: *Calotropis procera*, phytochemicals, stem bark, seed, leaf

1. Introduction

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because even the plants that manufacture them may not require them. Any part of the plant body may contain phytochemicals though the quantity and quality of phytochemicals present in plant parts may differ from one part to another [1]. Phytochemicals are naturally occurring bioactive substances in the medicinal plants, leaves, seeds, flower, vegetables and roots that have defense mechanism and protect from various diseases. Some are responsible for color and other organoleptic properties, such as the smell of garlic. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients [2].

Successful extraction, determination and isolation of biologically active components from plant material are largely dependent on the type of solvent [1] used in the extraction procedure. This therefore calls for the need to try various solvents to determine the percentage yield of extracts and screen the extracts from the plant parts for phytochemicals to ascertain the suitable solvent(s).

Calotropis procera is a species of flowering plant in the dogbane family, Asclepiadaceae [3]. It grows in the tropical rainforest region of Nigeria and sometime savanna areas [4]. It is locally called "Bomu bomu" (Yoruba), "Tumifafiya" (Hausa) [5]. It is a xerophytic, erect shrub, growing widely throughout the tropical and sub-tropical regions of Asia and Africa. Nature has been a supplier of medicines and medicinal agents for years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants have been a cure for human and animal diseases as they contain phytochemicals of therapeutic value [2]. Different parts (root, stem, leaves, flowers and seeds) of *Calotropis procera* are traditionally used to cure a number

of diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea [6].

It is a traditional medicinal plant [7] with unique properties [8]. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide [9]. The main objective of this research work was to analyze the various solvent extracts obtained from the leaf, seed and stems bark of *Calotropis procera* and qualitatively screened them for phytochemicals using standard tests.

2. Materials and Methods

2.1 Sample Collection and identification

Fresh leaves, stem barks and fruits of *Calotropis procera* were obtained from Odo-Ado Area, Ado Ekiti, Ekiti state, Nigeria. The plant materials were transported in polythene bags to the chemistry laboratory of Department of Science Technology, The Federal Polytechnic Ado-Ekiti where they were identified by Mr. Komolafe O.D. (an organic & natural product chemist) and Dr Ajibade V.A.(Biology & Microbiology Unit, The Federal, Polytechnic, Ado Ekiti).

2.2 Sample preparation

The fresh leaves, stem barks and seeds of *Calotropis procera* were properly cleaned to remove any dirt or filthy particles present on the surface. The pieces of plant materials were air dried for 16 days. The dried plant materials were taken separately and crushed using an electric blender to obtain a fine powder and sieve to obtain finer particles. The powdered samples were stored in clean plastic containers until needed for analysis.

2.3 Solvent Extraction

Solvent: Ethanol, Diethyl Ether, Chloroform, Methanol, N-hexane, De ionized water, Ethyl Acetate, Acetone, Petroleum Ether, Isopropanol

Method – Maceration

2.4 Procedure

10g portion of each powdered plant part materials was weighed with weighing balance and soaked in 150ml of each solvent at room temperature for 48 hrs with vigorous shaking at regular interval. The materials were filtered first with muslin cloth and then with filter paper. The filtrates were concentrated using Rotary Evaporator and the concentrates were used for the phytochemicals screening using standard procedures.

2.5 Phytochemicals Screening

Test for Alkaloids (Wagner's test)

A portion of extract was treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodine in 100ml of water) and observed for the formation of reddish brown precipitate

Test for Carbohydrate (Molish's test)

Drops of Molish's reagent were added to 2ml portion of the extract. This was followed by addition of 2ml of conc.H₂SO₄ down the side of the test tube. The mixture was then allowed to stand for 2-3 minutes. Formation of a red or dull violet colour at the interphase of the two layers gave a positive test.

Test for Cardiac glycosides (Keller Kelliani's test)

5ml of extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underplayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Test for Flavonoid (Alkaline reagent test).

2ml of extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour,

which becomes colourless on addition of dilute hydrochloric acid indicate the presence of flavonoid.

Test for Phenols (Ferric chloride test)

A portion of the extract was treated with aqueous 5% ferric and observed for formation of deep blue or black colour.

Test for Amino acid and protein (1%ninhydrin solution in acetone)

2ml of extract was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minute and observed for the formation of purple colour.

Test for Saponins (Foam test)

To 2ml of extract was added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Sterols (Liebermann- burchard test)

1ml of extract was treated with drops of chloroform, acetic anhydride and conc.H₂SO₄ and observed for the formation of dark pink or red colour.

Test for Tannins (Braymer's test)

2ml of extract was treated with 10%alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for Terpernoids (Salkowki's test)

1ml of chloroform was added to 2ml of each extract followed by a few drops of conc. sulphuric acid. A reddish brown precipitate produce immediately indicate the presence of terpenoids.

Test for Quinones

A small portion of extract with conc.HCL and observed for the formation of yellow precipitate

Test for Oxalate

To 3ml portion of extract were added a few drops of ethanoic acid glacial. A greenish black colouration indicates the presence of oxalate.

3. Result and Discussion

Table 1: Result of Phytochemical screening of *Calotropis procera* bark extract.

Solvent	%yield	Alkaloid	Saponin	Flavonoid	Terpanoid	Glycoside	Tanin	Phenolic compound	Carbohydrate	Amino acid	Quinones	Oxalate	Sterol
Acetone	5.43	+	-	-	-	+	-	-	-	-	+	-	-
Chloroform	3.42	+	+	-	+	+	-	-	+	-	+	+	-
Diethyl ether	2.74	+	+	-	+	+	-	-	+	-	+	-	-
Ethanol	3.33	-	+	-	+	+	-	-	+	-	+	-	-
Ethyl acetate	3.83	+	-	-	+	+	-	-	+	-	-	-	-
Isopropanol	2.36	+	+	-	+	-	+	-	+	-	-	-	-
Methanol	2.2	+	-	-	+	+	-	-	-	-	-	-	-
N-hexane	0.76	-	+	+	+	+	-	-	+	-	-	-	-
Pet. Ether	2.18	-	+	-	-	-	-	-	-	+	+	-	-
Water	5.62	+	+	-	-	+	-	+	+	-	+	-	-

+ = present - = absent

Table 2: Result of Phytochemical screening of *Calotropis procera* seed extract.

Solvent	% yield	Alkaloid	Saponin	Flavonoid	Terpanoid	Glycoside	Tanin	Phenolic compound	Carbohydrate	Amino acid	Quinones	oxalate	Sterol
Acetone	6.87	+	+	-	+	+	-	-	-	-	-	-	+
Chloroform	10.18	-	+	+	-	+	-	-	+	-	-	-	+
Diethyl ether	3.95	+	+	-	+	+	-	-	+	-	+	-	+
Ethanol	4.27	+	-	-	+	+	-	-	+	-	+	-	+
Ethyl acetate	2.58	+	-	+	+	+	-	-	+	-	-	-	-
Isopropanol	7.79	+	-	+	+	+	-	-	+	-	-	-	-
Methanol	7.91	+	+	+	-	+	-	-	+	+	+	-	+
N-hexane	6.05	-	-	+	+	-	-	-	+	-	-	-	-
Pet. Ether	9.15	+	+	-	-	+	-	-	+	+	-	-	-
Water	1.7	+	++	-	+	+	-	+	+	-	-	-	-

+ = present - = absent

Table 3: Result of Phytochemical screening of *Calotropis procera* leaf extract.

solvent	% yield	Alkaloid	saponin	Flavonoid	terpanoid	Glycoside	tanin	Phenolic compound	carbohydrate	Amino acid	quinones	oxalate	Sterol
Acetone	9.77	+	+	-	-	+	-	-	-	-	-	-	-
Chloroform	3.61	+	+	-	+	-	+	-	+	-	-	+	+
Diethyl ether	2.53	-	+	-	-	-	+	-	-	-	-	+	-
Ethanol	6.62	+	+	-	-	+	+	+	+	-	+	-	-
Ethyl acetate	5.58	-	-	-	-	+	-	+	-	-	-	+	-
Isopropanol	2.38	+	+	+	+	+	+	-	-	-	-	+	+
Methanol	8.62	+	+	+	-	+	+	+	+	+	+	-	+
N-hexane	1.02	-	+	+	-	+	-	-	-	-	-	-	-
Pet. Ether	0.83	-	-	-	+	+	-	-	-	+	+	-	+
Water	5.21	+	-	-	-	+	+	+	+	-	+	-	-

+ = present - = absent

Results obtained for the qualitative phytochemicals screening of various solvent extracts of stem bark of *calotropis procera* are presented in table I. Of the twelve phytochemicals screened for, eleven were found present in one solvent extract or the other. They are alkaloid, saponin, flavonoid, terpenoid, glycosides, tannin, phenolic compounds, carbohydrate, amino acid, quinines and oxalate. None of the solvent extracts had the presence of sterol. Based on %yield of solvent extracts, water had the highest %yield while n-hexane had the least %yield.

Results obtained for the qualitative phytochemicals screening of various solvent extracts of seed of *calotropis procera* are presented in table II. Of the twelve phytochemicals screened for, ten were found present in one solvent extract or the other. They are alkaloid, saponin, flavonoid, terpenoid, glycosides, phenolic compounds, carbohydrate, amino acid, quinines and sterol. None of the solvent extracts had the presence of tannin and oxalate. Based on %yield of solvent extracts, chloroform had the highest %yield while water had the least %yield.

Results obtained for the qualitative phytochemicals screening of various solvent extracts of leaf of *calotropis procera* are presented in table III. All the twelve phytochemicals screened for were found present in one solvent extract or the other. They are alkaloid, saponin, flavonoid, terpenoid, glycosides, tannin, phenolic compounds, carbohydrate, amino acid, quinines, oxalate and sterol. Based on %yield of solvent extracts, acetone had the highest %yield while petroleum ether had the least %yield.

4. Conclusion

Nature has been a supplier of medicines and medicinal agents for years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants have been a cure for human and animal diseases as they contain phytochemicals of therapeutic value [10]. To achieve this, Successful extraction, determination and isolation of biologically active components from plant material is largely dependent on the type of solvent used in the extraction procedure. The outcome of this research work indicate that water is a good solvent for the extraction of alkaloid, glycosides, saponin, terpenoid and carbohydrate as it was able to to extract them from the three plant parts. N-hexane is a good solvent for the extraction of flavonoid, chloroform is a good solvent for saponin and carbohydrate, diethyl ether is a good solvent for saponin, ethanol is a good solvent for the extraction of alkaloid, glycosides, carbohydrate and quinines, methanol is a good solvent for the extraction of glycosides, flavonoid, tannin, phenolic compounds and amino acid, petroleum ether is a good solvent for the extraction of amino acid, isopropanol is a good solvent for the extraction of alkaloid and terpenoid, and acetone is a good solvent for the extraction of alkaloid and glycosides. Also the presence of different phytochemicals in the plant parts (leaf, seed and stem bark) of *calotropis procera* further confirm the ability of the plant to serve a source of natural medicines. Furthermore, isolation, purification and quantification of the phytochemicals found present will allow scientists value from the plant.

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