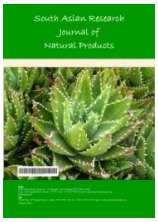

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# Bioassay Guided Fractionation, Phytochemicals and Toxicity Evaluation of *Eucalyptus camaldulensis* Leave Extracts

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author YA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TMA, IAM and MM managed the analyses of the study. Authors MMD, LAA, SAB and SIS managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Medicinal plants have found a wide range of application in pharmacy, medicine, and toxicology, and are very valuable tool for their bioassay guided fractionation. About 70% of huge development efforts from medicinal plant centers are on the safety of such plants in laboratory or experimental animal as well human Leaves of *E. camaldulensis* are known to possess many biological and pharmacological effect for treatment of many diseases. and it has been reported to have antioxidants, cytotoxic effects, antimicrobial and anti-dermatophytes properties. This study is aimed to determine the phytochemical content and acute toxicity study of *Eucalyptus camaldulensis* leave extract using different solvent of varying polarity.
**Methods:** Bioassay Guided Fractionation of Aqueous Leave Extract of *Eucalyptus camaldulensis* were carried out and Sequentially fractionated using hexane, ethyl acetate and chloroform. The acute toxicity study LD50 (oral rats) was evaluated according to Muhammad [1] while the phytochemicals screening was conducted using the methods described by Brain and tuner [2].

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**Results.** The percentage yield of the four solvent extract of *Eucalyptus camaldulensis* leaves extract showed that ethyl acetate extract has the highest yield followed by aqueous extract. While hexane and chloroform extract showed low yield when compared with the ethyl acetate and aqueous extract. The phytochemical screening of *Eucalyptus camaldulensis aqueous, hexane, ethyl acetate and chloroform leave* extracts shows the presence of tannins, saponins, Flavonoids, Cardiac Glycosides, Steroids, Terpenoids and Anthraquinones. However, saponins, flavonoids steroids and Terpenoids were absent in chloroform, hexane and ethyl acetate, hexane and aqueous extract respectively, while alkaloids were found to be absent in all the four solvent extracts. The acute lethal study of aqueous, hexane, ethyl acetate and chloroform extract of *Eucalyptus camaldulensis* Leaves were found to be greater than 5000 mg/kg body weight.
**Conclusion and Recommendation:** The finding showed that *Eucalyptus camaldulensis* leave extract possess some bioactive constituents of pharmacological important and all the extracts such as aqueous, hexane, chloroform and ethyl acetate were found to be practically non-toxic to the experimental animals It is recommended that further studies should be explored for isolation of the active ingredient for drugs discovery.

*Keywords: Eucalyptus camaldenensis; leave; phytochemicals; LD50.*

## 1. INTRODUCTION

Traditional medicine needs to be evaluated, given due recognition and development so as to improve its efficiency, safety, availability and wider application at low cost [3]. The use of plants or their products in the prevention and treatment of disease is known for years [4]. Medicinal plants have served as rich sources of pharmacologically active substances. Herbs have been used in a diverse array of purposes, including medicine, nutrition, flavoring, dying, repellents, fragrances, cosmetic, charms, smoking and industrial uses. Today, herbs are still found in 40% of prescription drugs [5]. Most or all plants have medical use, which are referred to as herbal medicine, and are needed for therapeutic and medicinal use [6].

Medicinal plants have found a wide range of application in pharmacy, medicine and toxicology and are very valuable tool for their bioassay guided fractionation [6]. About 70% studies from medicinal plant focusses on the safety of the plants [7]. Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another. Most reports concerning the toxic effects of herbs medicines are associated with hepatotoxicity although reports of other toxic effects including kidney (nephrotoxicity), nervous system, blood, cardiovascular and dermatologic effects, mutagenicity and carcinogenicity have also been published in the medical literature [8]. The actual dose of active compounds being consumed is often variable, unpredictable or simply unknown. When compared with adults, children may be particularly susceptible to the toxic effects of such dosage, variation due to their smaller size and different capacity for detoxifying chemicals [8].

**Phytochemicals:** Phytonutrients work in a number of different ways, depending on the specific substance. Many are antioxidants and help to protect against cancer risks and other cell damages. This includes the carotenoids which are found in carrots and many fruits, the polyphenols which are abundant in grapes and certain types of teas, the allyl sulfides contained in garlic, onions, and leeks, and the flavonoids which fruits and vegetables are known for. Other phytochemicals like iso-flavones which are found in soy products help with the action of hormones in the body. Other chemicals in plants can be natural anti-bacterials, including allicin, which is found in garlic. Still others can have a physical action on the body. Proanthocyanidins are phytonutrients found in cranberries, bind to the cells so that bacteria can not stick to the urinary tract as easily [7]. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [9]. Many of these indigenous medicinal plants are used as spices and food plants. They are sometimes added to foods for pregnant and nursing mothers for medicinal purposes [10].

*Eucalyptus camaldulensis* usually called “red river gum” is a tree of the genus *Eucalyptus*. It is a plantation species in many parts of the world

including Nigeria but it is native to Austria where it is widespread especially beside inland water courses. The leaves of *Eucalyptus camaldulensis* in particular are evergreen. The tree is about 24 – 40 m high with stout trunk, the bark is smooth, white grey or buff. The plant has widespread application as a medicinal plant. In Nigeria and some parts of sub – Saharan Africa, its medicinal uses include the use of its oil as a remedy for cough and cold. The gum when boiled with water and sugar, become a liquid drink used to treat pulmonary complaints and as a general anesthetic for tooth ache. An infusion of the bark is used for management of diarrhea [11]. This is study is aimed to determine the phytochemical content and acute toxicity study of *Eucalyptus camaldulensis* leave extract using different solvent of varying polarity.

## 2. METHODS

### 2.1 Collection and Identification

The *Eucalyptus camaldulensis* leave was collected from Wudil Local government area, Kano state, Nigeria. It was authenticated at Biological Sciences Department, Kano University of Science and technology, Wudil, Kano, Nigeria. And was identified as *Eucalyptus camaldulensis* deposited at the herbarium with a number EC20218.

### 2.2 Preparation of Extract

The leave of *Eucalyptus camaldulensis* was washed with clean water and dried under closed laminar flow hood, after which it was pulverized to coarse powder using mechanical grinder. *Eucalyptus camaldulensis* aqueous leave extract was prepared according to Gafna et al. [6], method. One thousand grams (1000 g) of the powder *Eucalyptus camaldulensis* leave was mixed and soaked in 2000 $cm^3$ distilled water in a 2 litre conical flask, the content of the flask was mixed vigorously. The mixture was shaken and top covered with aluminum foil and kept for 48 hours. The aqueous extract was obtained by filtration using whatman No1 filter paper and concentrated using vacuum evaporator at 60°C in water bath (OSL200 water bath and shaker Grand instrument, Cambridge). The concentration and total yield of the aqueous leave extract of *Eucalyptus camaldulensis* was determined using the below formulae and stored in air tied container for further analysis.

### 2.3 Bioassay Guided Fractionation of Aqueous Leave Extract of *Eucalyptus camaldulensis* and Sequential Fractionation of the Aqueous Leave Extract of *Eucalyptus camaldulensis* Using Solvent of Varying Polarity

The Aqueous leave Extract of *Eucalyptus camaldulensis* was sequentially partitioned with solvent of varying polarity this include hexane, chloroform and ethyl acetate.300 g of aqueous leave extract was mixed with 1000 ml hexane, mixed and shake, it was allowed to stand overnight with intermittent stirring, the hexane extract was collected. On the same aqueous extract, the procedure was repeated with chloroform follow by ethyl acetate and the total yield of each extracts was determined using the formular below.

$$\text{Percentage yield(\% w/w)} = \frac{\text{Weight of the sample extract obtained (g)}}{\text{Weight of the powdered sampled used (g)}} \times 100$$

### 2.4 Acute Toxicity Study ($LD_{50\ (ORAL,\ RATS)}$) of *Eucalyptus camaldulensis* Leave Extracts

The $LD_{50(Oral,\ rats)}$ were determined by Muhammad et al. [1]. This was carried out in two phases, in the first phase the rats were divided into three groups of three rats each and were administered with 10, 100 and 1000 mg/kg of all the four solvents leave extracts of *Eucalyptus camaldulensis* orally. The rats were observed for mortality and general behavior. In the second phase, the rats were group into five group of one rat each and were administered with all the four leave extracts of *Eucalyptus camaldulensis* at varying doses of 1250, 2000, 2750, 3750 and 5000 mg/kg. The rats were observed for 24 hours for mortality and other signs of toxicity.

### 2.5 Qualitative Phytochemical Screening of *Eucalyptus camaldulensis* Leave Extracts

The standard techniques described by Tiwari et al. [12] was adopted in this research, in which the concentrated extracts of the pulverized leave were screened for Phytochemicals such as alkaloids, tannins, saponins, glycosides, steroids/triterpenoids, flavonoids and Anthraquinones.

#### 2.5.1 Test for alkaloid

0.5 ml of leaf oil was added to 5 $cm^3$ 1% aqueous HCl and was stirred. A few drops of

Dragren droffs reagent (potassium bismuth iodide solution) was added, and 1 $cm^3$ portion of the solution formed was treated with wagners reagent (solution of iodine in potassium iodide). A white precipitate formed indicated the presence of Alkaloids. This method was adopted from the work of Gul et al. [13].

### 2.5.2 Test for tannins

The method described by Basumatary et al. [14] was adopted in this research. 0.5 ml of oil was boiled in 20 $cm^3$ of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and brownish green color observed indicated the present of tannin.

### 2.5.3 Test for saponins

The method described by Basumatary et al. [14] was adopted in this research. 2 ml of the oil was boiled in 20 $cm^3$ of distilled water in a water bath and filtered, and 10$cm^3$ of the filtrate was mixed with 5 $cm^3$ of distilled water and shaken vigorously until stable persistent froth formed. The frothed was mixed with 3 drops of olive oil and shaken vigorously and an emulsion observed indicted the present of saponins.

### 2.5.4 Test for flavonoids

The method described by Kumar et al. [15] was adopted in this research. 5 $cm^3$ of dilute ammonia solution was added to a portion of the filtrate obtained from the extract above and concentrated $H_2S0_4$ was added. A yellow colouration observed which disappeared on standing indicated the presence of flavonoids.

### 2.5.5 Test for terpenoids

Salkowski method was adopted in this research. To 5 $cm^3$ of the oil, 2 $cm^3$ of chloroform was added and 3 $cm^3$ concentrated $H_2S0_4$was carefully added and a reddish brown coloration at the interface of the layer formed indicated the presence of terpenoids.

### 2.5.6 Test for cardiac glycosides

According to Keller-Killani test/method adopted [16] 5 $cm^3$ of oil was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution under layed with 1 ml of concentrated sulphuric acid. A brown ring at the interface observed indicates the presence of deoxysugar, characteristic of cardenolides.

## 2.6 Statistical Analysis

The data was statistically analyzed at P-value ($p<0.05$) significantly accepted and a comparison between the groups was performed using one-way analysis of variance (ANOVA) by Graphpad instat3 software (2000) version 3.05 by Graphpad Inc. The data are given as the mean ± standard deviation.

## 3. RESULTS AND DISCUSSION

## 3.1 Total and Percentage Yield of *Eucalyptus camaldulensis* Leaves

The percentage yield of *Eucalyptus camaldulensis* leaves (1000 g) of all the four solvents extract were evaluated after 24 hours percolation. The percentage yield of Hexane, chloroform, ethyl acetate and aqueous extracts were found to be 1.40%, 4.69%, 9.29% and 7.30% respectively (Table 1).

The percentage yield of the four solvent extract of *Eucalyptus camaldulensis* leaves extract showed that ethyl acetate extract has the highest yield followed by aqueous extract. While hexane and chloroform extract showed low yield when compared with the ethyl acetate and aqueous extract. The differences in the extraction yields of *Eucalyptus camaldulensis* leaves may be due to varied chemical composition of the leaves [17]. And it may be associated with salvation of antioxidants compound(s) due to interactions of hydrogen bonds between the polar sites of the compound(s) present and the solvent [17].

**Table 1. Total yield and percent yield of aqueous, ethyl acetate, hexane and chloroform extracts of *Eucalyptus camaldulensis* leaves after 24 hours percolation**

| No. | Solvent of extract | Initial weight of plant powder (gm) | Final weight(g) of plant extract (gm) | Yield (%)W / W |
|---|---|---|---|---|
| **1** | Hexane | 70 | 0.982 | 1.40 |
| **2** | Chloroform | 70 | 3.283 | 4.69 |
| **3** | Ethylacetate | 70 | 6.503 | 9.29 |
| **4** | Water | 60 | 4.380 | 7.30 |

### 3.2 Qualitative Phytochemicals Screening of *Eucalyptus camaldulensis* Leave Extracts

The phytochemical screening of *Eucalyptus camaldulensis* aqueous, hexane, ethyl acetate and chloroform leave extracts shows the presence of tannins, the tannins were found to be extremely high in aqueous extract (Table 2). Tannins were known to show good medicinal properties and have exhibited good physiological activity. And have strong anti-microbial effects [18].

Saponins were present in aqueous, hexane and ethyl acetate but absent in chloroform extract (Table 2). Saponins served as expectorant and emulsifying agents as well it has good antifungal activity [18]. Alkaloids were found to be absent in all the four extract of *Eucalyptus camaldulensis* leaves.

Flavonoids were found moderately in aqueous extracts and found in small amount in chloroform extract of *Eucalyptus camaldulensis leaves.* However, it was found absent in hexane and chloroform extract. While cardiac glycoside was found extremely in all the four solvents extract of *Eucalyptus camaldulensis* leaves. Flavonoids and glycoside were extensively used for management of many diseases hence their usage in herbal medicine [17].

The aqueous, hexane and ethyl acetate contains steroid but absent in chloroform extract (Table 2). This indicated that the presence of steroids in *Eucalyptus camaldulensis* leave proved the important and the emphasis of it pharmaceutical role in the development of sex hormone and other reproductive related compounds [10].

Terpenoids were absent in aqueous extract but extremely found in ethyl acetate followed by chloroform than hexane extract. Terpenoids was reported to have antimicrobial, antihyperglycemics and immunomodulatory effect [19].

Anthraquinones were found extremely in all the four solvent extract of *Eucalyptus camaldulensis* leave extract (Table 2). Anthraquinones is extensively used to prevent plant from many diseases and possess strong antimicrobial activity [20].

### 3.3 Acute Toxicity Evaluation

The acute lethal study of aqueous, hexane, ethyl acetate and chloroform extracts of *Eucalyptus camaldulensis* Leaves (Table 3 to 4) shows that no any mortality within 24 hours oral administration of the extracts in both phase one and two and the LD50 oral rats was found to be greater than 5000 mg/kg body weight.

Muhammad [1] detailed that $LD_{50}$ values may be measured precisely and reproductively, the information of its correct numerical esteem would scarcely be of essentially critical, since an extrapolation from the experimental animals to human may not be conceivable. Be that as it may, it serves as to begin with guides to the security or toxic potential of a substance whose harmfulness profile isn't known [21].

Liver is the major site of detoxification of drugs and toxins [22]. Some medicinal plants are toxic to the liver and kidney [23]. The $LD_{50}$ for a particular substance is essentially the amount that can be expected to cause death in half (i.e.50%) of a group of some particular animal species, usually rats or mice. It is usually expressed as the amount of chemical administered (eg. Milligrams) per 100 grams (for small animals) or per kilogram (for bigger subjects) of the body weight of the test animal [24]. $LD_{50}$ obtained at the end of the study is reported in relation to the route of administration

**Table 2. Qualitative phytochemicals screening of *Eucalyptus camaldulensis* leaves**

| Phytochemical | Aqueous | Hexane | Ethylacetate | Chloroform |
|---|---|---|---|---|
| Tannins | + + + | + + + | + + + | + + + |
| Saponins | + | + | + | - |
| Alkaloids | - | - | - | - |
| Flavonoids | ++ | - | - | + |
| Cardiac Glycosides | + + + | + + + | + + + | + + + |
| Steroids | + + + | - | + + + | + + + |
| Terpenoids | - | + | ++++ | ++ |
| Anthraquinones | + + + | + + + | + + + | + + + |

*key: + =Trace, ++ = Moderate , +++ = High , ++++ = Very High, - = Absent*

**Table 3. First phase $LD_{50}$ (Oral, rat) of aqueous and ethyl acetate stem bark extracts of *Eucalyptus camaldulensis***

| Doses in (mg/kg) | Aqueous extract | | Ethyl acetate extract | |
|---|---|---|---|---|
| **Phase I** | **No: of Rat** | **Mortality** | **No: of Rat** | **Mortality** |
| 10 | 3 | 0/3 | 3 | 0/3 |
| 100 | 3 | 0/3 | 3 | 0/3 |
| 1000 | 3 | 0/3 | 3 | 0/3 |
| **Phase II** | | | | |
| 1250 | 1 | 0/1 | 1 | 0/1 |
| 2000 | 1 | 0/1 | 1 | 0/1 |
| 2750 | 1 | 0/1 | 1 | 0/1 |
| 3750 | 1 | 0/1 | 1 | 0/1 |
| 5000 | 1 | 0/1 | 1 | 0/1 |

*$LD_{50}$ Oral, rats of aqueous and ethyl acetate stem bark extracts of Eucalyptus camaldulensis is > 5000 mg/kg body weight of rats*

**Table 4. First phase $LD_{50}$ (Oral, rat) of hexane and chloroform stem bark extracts of *Eucalyptus camaldulensis***

| Doses in (mg/kg) | Hexane extract | | Chloroform extract | |
|---|---|---|---|---|
| **Phase I** | **No: of Rat** | **Mortality** | **No: of Rat** | **Mortality** |
| 10 | 3 | 0/3 | 3 | 0/3 |
| 100 | 3 | 0/3 | 3 | 0/3 |
| 1000 | 3 | 0/3 | 3 | 0/3 |
| **Phase II** | | | | |
| 1250 | 1 | 0/1 | 1 | 0/1 |
| 2000 | 1 | 0/1 | 1 | 0/1 |
| 2750 | 1 | 0/1 | 1 | 0/1 |
| 3750 | 1 | 0/1 | 1 | 0/1 |
| 5000 | 1 | 0/1 | 1 | 0/1 |

*$LD_{50}$ Oral, rats of hexane and chloroform leave extract of Eucalyptus camaldulensis is > 5000 mg/kg body weight of rats*

of the test substance e.g $LD_{50}$ (oral), $LD_{50}$ (dermal) etc. The most frequently performed lethal study is the oral $LD_{50}$. Results obtained from oral studies are important for drugs, food and accidental domestic poisonings [24].

## 4. CONCLUSION

The finding showed that ethyl acetate extract has the highest yield followed by water, chloroform and hexane extracts. The *Eucalyptus camaldulensis* leave extract possess some bioactive constituents of pharmacological important and all the extracts such as aqueous, hexane, chloroform and ethyl acetate were found to be practically non-toxic to the experimental animals.

## ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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